REVIEW

IGF-binding protein-4: biochemical characteristics and functional consequences

R Zhou^{1, 2}, D Diehl¹, A Hoefflich¹, H Lahm³ and E Wolf¹

¹Institute of Molecular Animal Breeding and Biotechnology, Gene Center of the University of Munich, 81377 Munich, Germany

²College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, 430070 Wuhan, China

³Immunology–Molecular Biology Laboratory, Thoraxklinik Heidelberg gGmbH, 69126 Heidelberg, Germany

(Requests for offprints should be addressed to R Zhou; Email: zhou@lmb.uni-muenchen.de)

Abstract

IGFs have multiple functions regarding cellular growth, survival and differentiation under different physiological and pathological conditions. IGF effects are modulated systemically and locally by six high-affinity IGF-binding proteins (IGFBP-1 to -6). Despite their structural similarity, each IGFBP has unique properties and exhibits specific functions. IGFBP-4, the smallest IGFBP, exists in both non-glycosylated and N-glycosylated forms in all biological fluids. It is expressed by a wide range of cell types and tissues, and its expression is regulated by different mechanisms in a cell type-specific manner.

IGFBP-4 binds IGF-I and IGF-II with similar affinities and inhibits their actions under almost all *in vitro* and *in vivo* conditions. In this review, we summarize the available data regarding the following aspects of IGFBP-4: genomic organization, protein structure–function relationship, expression and its regulation, as well as IGF-dependent and -independent actions. The biological significance of IGFBP-4 for reproductive physiology, bone formation, renal pathophysiology and cancer is discussed.

Journal of Endocrinology (2003) **178**, 177–193

Introduction

Insulin-like growth factors (IGF-I and IGF-II), two growth-promoting peptides, have both mitogenic and metabolic actions that are involved in growth, survival and differentiation of many cell types and tissues under different physiological and pathological situations (reviews: Cohick & Clemmons 1993, Stewart & Rotwein 1996). IGFs can act in both an endocrine and a paracrine/autocrine manner (reviews: Cohick & Clemmons 1993, Mohan *et al.* 1996, Stewart & Rotwein 1996, Butler & LeRoith 2001). Gene-targeting studies in mice have demonstrated that both IGF-I and IGF-II are essential for growth and development (Liu *et al.* 1993, Liu *et al.* 1998, Butler & LeRoith 2001).

The IGFs interact with specific cell surface receptors, designated type I and type II IGF receptors (IGF-IR and IGF-IIR). Most of the actions of IGF-I and IGF-II are mediated by the IGF-IR, which is a transmembrane receptor with tyrosine kinase activity (reviews: LeRoith *et al.* 1995, LeRoith 2000, De Meyts & Whittaker 2002). IGF-IIR binds IGF-II with high affinity but interacts minimally with IGF-I (review: Bralke 1999). Gene-

targeting studies revealed that IGF-IIR is important for the control of embryonic growth, and for internalization and degradation of extracellular IGF-II (reviews: Bralke 1999, Hassan 2003); however, it is unclear whether this receptor is involved in IGF-II signaling. IGF-IIR is identical to the cation-independent mannose-6-phosphate (Man-6-P) receptor that is involved in transport of Man-6-P-bearing lysosomal enzymes from their sites of synthesis into an endosomal/pre-lysosomal compartment (reviews: Bralke 1999, Hassan 2003).

The IGFs in serum and other extracellular environments are bound to specific IGF-binding proteins (IGFBPs), which represent a family of six secreted proteins with a common domain organization. They all have an N-terminal domain with 12 conserved Cys residues, a C-terminal domain with six conserved Cys residues, and a central (L) domain with no Cys residues except in IGFBP-4 (review: Duan 2002). The N- and C-domains are at least 50% homologous among the six IGFBPs in a given species, and for each IGFBP there is roughly 80% homology among different vertebrate species. The L-domain shows little similarity between species (reviews: Rechler 1993, Kelley *et al.* 1996). Since the affinities of

IGFBPs for the IGFs are equal to or greater than those of the IGF receptors, several mechanisms have evolved which decrease IGFBP affinities and increase IGF bioavailability to the receptors. These mechanisms include phosphorylation, glycosylation, proteolysis and the adherence to the cell surface or extracellular matrix (ECM) (reviews: Jones & Clemmons 1995, Clemmons 1997).

Recently, several so-called IGFBP-related proteins (IGFBP-rPs) have been discovered, which exhibit structural homology to the N-terminal region of the classical IGFBPs, but have substantially lower affinities for IGFs (review: Hwa *et al.* 1999). The functional significance of the IGFBP-rPs for the IGF system is currently unclear.

IGFBPs have a plethora of functions. In addition to acting as carrier proteins, IGFBPs have been shown to inhibit or potentiate IGF actions. In serum and other biological fluids, IGFBPs modulate the endocrine actions of IGFs by regulating the bioavailability of IGFs for their receptors. IGFBPs are also expressed locally in a broad spectrum of tissues and act as autocrine/paracrine regulators of IGF effects. Furthermore, some IGFBPs have been demonstrated to have IGF-independent actions (reviews: Murphy 1998, Wetterau *et al.* 1999, Mohan & Baylink 2002).

Among the six IGFBPs, IGFBP-4 is the smallest and is unique in that it has been consistently shown to inhibit IGF actions (Wetterau *et al.* 1999). IGFBP-4 was first described on the basis of its ability to potently inhibit bone cell growth (Mohan *et al.* 1989) and follicle-stimulating hormone-stimulated steroid production of ovarian granulosa cells (Ui *et al.* 1989). The most likely mechanism is binding of secreted IGFs, preventing their interaction with IGF receptors (Mohan *et al.* 1995b). However, possible IGF-independent pathways of IGFBP-4 action have also been discussed (Singh *et al.* 1994, Perks *et al.* 1999, Wright *et al.* 2002).

In this review, we summarize the present knowledge of the genomic organization of the IGFBP-4 gene, structure–function relationships of IGFBP-4, IGFBP-4 expression and its regulation, as well as the IGF-dependent and -independent actions of IGFBP-4. The biological significance of IGFBP-4 is also discussed.

Genomic organization of the IGFBP-4 gene

The human *IGFBP4* gene is located on chromosome 17 (Allander *et al.* 1993) and spans about 15.3 kb (Zazzi *et al.* 1998). According to the mouse genome sequence determined so far, the mouse *Igfbp4* gene spans 11.3 kb on chromosome 11 (<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=16010>). The rat *Igfbp4* gene spans at least 12 kb of genomic sequence (Gao *et al.* 1993). The genes for human IGFBP-4 (hIGFBP-4) and rat IGFBP-4 (rIGFBP-4) are composed of four exons separated by three introns, which give them an arrangement similar to the genes of the other IGFBPs except for

IGFBP-3 (Cubbage *et al.* 1990). The splice sites are highly conserved between human *IGFBP4* and rat *Igfbp4* genes, but the sizes of the introns vary slightly between the two species (Zazzi *et al.* 1998). Cell type-specific transcript sizes were documented in mouse cell lines which, when translated, suggest an additional non-IGF-binding variant present in mouse cells (Glantschnig *et al.* 1998).

Alignment of the published rat (Gao *et al.* 1993), human (Dai *et al.* 1997, Zazzi *et al.* 1998) and mouse (Glantschnig *et al.* 1998) IGFBP-4 promoter sequences revealed an overall high evolutionary conservation, but some promoter regions show less conservation and vary between the three species. It is interesting that the human sequence differs from rodent sequences by a 12 bp insertion upstream to the transcription initiation codon (Dai *et al.* 1997). The IGFBP-4 promoter possesses a typical TATA box and a CAAT box. Several potential regulatory elements, such as cAMP responsive elements, steroid responsive elements, AP-1-binding sites and Sp1-binding sites exist in the IGFBP-4 5'-flanking regions of the three species (Gao *et al.* 1993, Dai *et al.* 1997, Glantschnig *et al.* 1998, Zazzi *et al.* 1998). These *cis*-regulatory binding sites provide the targets for a variety of local and systemic factors such as cAMP, parathyroid hormone (PTH) and various ligands of the steroid hormone receptor superfamily (such as glucocorticoids, retinoic acid, triiodothyronine, vitamin D), to regulate the expression of IGFBP-4 as discussed below.

Several *Alu* repeat sequences are clustered in the proximity (upstream) of the human *IGFBP4* gene, with an average of one *Alu* sequence per kb (Zazzi *et al.* 1998), which is a higher frequency than the normal distribution in the human genome (Houck *et al.* 1979). This indicates that the *IGFBP4* gene is a hot spot for *Alu* integration. High-density *Alu* regions are often sites of genomic instability (Calabretta *et al.* 1982) and show a higher frequency of sequence polymorphism (Batzner & Deininger 2002). Apart from the *Alu* repeat sequences, several polymorphic microsatellites were found within the boundaries of the human *IGFBP4* gene (Zazzi *et al.* 1998). One of these was used as a marker to locate the hereditary breast–ovarian cancer gene (Tonin *et al.* 1993). Another highly polymorphic microsatellite was found in the first intron of the human *IGFBP4* gene (Zazzi *et al.* 1998).

A typical cleavage site for poly(A) was found at the 3'-end of the human *IGFBP4* gene; however, no conserved poly(A) addition signal was detected within the 30 bp upstream region. Nevertheless, within this region an AAAAAA and several AACAAA consensus sequences were found, which could form a degenerate poly(A) addition signal. The few described eukaryotic genes that do not contain a standard AAUAAA sequence are involved in alternative polyadenylation, but this does not seem to be the case for the human *IGFBP4* gene, since no variation in mRNA length has been reported and no alternate polyadenylation site was found within the *IGFBP4* gene (Zazzi *et al.* 1998).

Figure 1 Structural features of hIGFBP-4. Numbers indicate cysteines involved in disulfide linkages (numbering based on the mature IGFBP-4 sequence derived from the proIGFBP-4 sequence containing a 21 aa signal peptide; SWISS-PROT accession number P22692). The conserved N-terminal and C-terminal regions of hIGFBP-4 are shaded, and the non-conserved midregion is unshaded. Cysteines are indicated by vertical lines and disulfide linkages by horizontal lines. The N-glycosylation site is shown by a trident and the PAPP-A cleavage site by an arrow.

The protein: structure–function relationship

hIGFBP-4 contains 237 amino acids (aa), and rIGFBP-4 consists of 233 aa. IGFBP-4 contains an N-linked glycosylation site and commonly exists in biological fluids as a doublet: a 24 kDa non-glycosylated form and a 28 kDa glycosylated form (Wetterau *et al.* 1999). IGFBP-4 is unique among the six IGFBPs in having two extra Cys residues in the variable L-domain encoded by exon 2 (Landale *et al.* 1995), which are linked to each other (Fig. 1) (Chelius *et al.* 2001). These unique properties of IGFBP-4 may be responsible for the distinctive biological behavior of this binding protein, i.e. solely inhibitory actions and lack of cell surface association.

IGF binding

IGFBP-4 inhibits IGF actions by preventing the binding of IGFs to their receptors. The binding of IGFBP-4 to IGFs is essential for this inhibitory effect (Mohan *et al.* 1995b, Miyakoshi *et al.* 1999). The IGF-binding domain may represent the major structural determinant of the biological activity of IGFBP-4. Therefore, elucidation of the structural determinants of various IGFBPs in IGF binding is important to the general understanding of the biology of the IGF system and may shed light on how these different IGFBPs exhibit specific actions.

It is generally accepted that the IGF-binding site for the various high-affinity IGFBPs is located in the N-terminal region. Mutational analysis indicated that the IGF-binding activity of hIGFBP-4 is mainly determined by the N-terminal region Leu⁷²–Ser⁹¹, and to a lesser extent by the C-terminal region Cys²⁰⁵–Val²¹⁴ (Qin *et al.* 1998). Although the three-dimensional structure of most IGFBPs has not been determined, disulfide bridging in IGFBPs appears to be important for maintaining the secondary structure required for IGF binding, since all six IGFBPs contain conserved Cys residues in both the N-terminal and the C-terminal regions and reduced IGFBPs exhibit little or no IGF-binding activity (Landale *et al.* 1995, Qin *et al.* 1998, Neumann & Bach 1999). Direct evidence

was provided by disruption of the disulfide linkages in the N-domain of rIGFBP-3, which resulted in complete loss of IGF-binding ability (Hashimoto *et al.* 1997). There is evidence suggesting that the IGF-binding domain in hIGFBP-4 involves a hydrophobic motif (Leu⁷²–Met⁸⁰) located in the distal part of the conserved N-terminal region, and that the N-terminal Cys residues (Cys9 and Cys12) are more critical than the C-terminal Cys residues (Cys17 and Cys20) for IGF binding (Byun *et al.* 2001a). Eight disulfide linkages in rIGFBP-4 and hIGFBP-4 have been determined, four in the N-terminal (two of them are present in all six IGFBPs), three in the C-terminal (present in IGFBP-2 and -6 as well) and one in the midregion (Fig. 1) (Chelius *et al.* 2001).

Although the IGF binding is mainly determined by the N-terminal conserved Cys residues, the six conserved C-terminal Cys residues in IGFBP-4 are essential for high-affinity binding of IGFs (Qin *et al.* 1998, Standker *et al.* 2000, Byun *et al.* 2001a). There is also evidence that in IGFBP-3 (Spencer & Chan 1995) and IGFBP-2 (Forbes *et al.* 1998, Wang *et al.* 1988) the C-terminal region plays an important role in IGF binding. The six C-terminal Cys residues in IGFBP-4 are linked in the same manner as in IGFBP-2 and -6 (Chelius *et al.* 2001). Both IGFBP-2 and -6 share a binding preference for IGF-II and have the same C-terminal disulfide linkages, suggesting that a different disulfide linkage could conceivably influence IGF-II binding preference (Forbes *et al.* 1998). However, IGFBP-4 binds IGF-I and IGF-II with similar affinities; thus the highly conserved three C-terminal disulfide linkages either have no effect on the IGF-binding preference, or they are not the sole determinants.

Cell surface association and tissue distribution

Cell surface and ECM association is one of the possible mechanisms to alter the affinity of IGFBPs for the IGFs. Both IGFBP-1 and IGFBP-2 have an Arg–Gly–Asp (RGD) motif, which was shown to bind $\alpha 5 \beta 1$ integrin and consequently to mediate cell surface association of IGFBP-1 (Jones *et al.* 1993). We demonstrated that IGFBP-2 bound to the plasma membrane also in the absence of the RGD motif in IGFBP-2 (Hoefflich *et al.* 2002), indicating that additional mechanisms are involved in cell surface association of IGFBP-2. IGFBP-2 can bind to heparin, ECM and proteoglycans depending on the previous binding to IGF molecules (Arai *et al.* 1996, Russo *et al.* 1999). IGFBP-3 and -5 bind to distinct membrane receptors (Oh *et al.* 1993, Andress 1995, 1998, Leal *et al.* 1997). However, there is no evidence for cell surface association of IGFBP-4 (Kelley *et al.* 1996), suggesting that IGFBP-4 exists primarily in a soluble extracellular form.

When IGFBP-3 was perfused through the isolated, beating rat heart, it crossed the microvascular endothelium

and was distributed primarily in cardiac muscle. In contrast, perfused IGFBP-4 also crossed the microvascular endothelium of the rat heart, but was preferentially distributed in connective tissue (Bar *et al.* 1990, Boes *et al.* 1992). A small basic C-terminal region (heparin-binding domain, HBD) of IGFBP-3 has been shown to be central to the ability of IGFBP-3 to bind to specific cells, such as endothelial cells (Booth *et al.* 1995, Knudtson *et al.* 2001). When this region was synthesized as an 18-mer peptide (P3), P3 bound to endothelial cells (Knudtson *et al.* 2001). IGFBP-4 lacks such an HBD and does not bind to endothelial cells (Booth *et al.* 1995). When the 20 C-terminal aa of IGFBP-4 region (P4) were replaced by the homologous P3 peptide, the generated chimeric IGFBP-4₃ bound specifically to endothelial cells, and it was distributed in the perfused rat heart similarly to the behavior of IGFBP-3 but different from that of IGFBP-4 (Knudtson *et al.* 2001), suggesting that the C-terminal region of IGFBP-4 is critical for its specific tissue distribution in the rat heart. These findings provide a novel potential mechanism of the tissue-specific actions of the IGFBPs.

Glycosylation

In 1991, Ceda and colleagues isolated two IGFBPs with apparent molecular weights (MW) of 28 and 24 kDa from the conditioned medium of B104 rat neuroblastoma cells (Ceda *et al.* 1991). Sequence analysis revealed that both proteins had identical N-terminal sequences and appeared to be two forms of IGFBP-4. Treatment of these IGFBPs with endoglycosidase-F reduced the MW of the 28 kDa IGFBP to 24 kDa. However, there was no change in the 24 kDa IGFBP. The data from this and other studies (Cheung *et al.* 1991, Carr *et al.* 1994) demonstrated that IGFBP-4 exists as both N-glycosylated and non-glycosylated protein. Further analysis revealed that a single N-linked glycosylation site is located in the midregion (Asn¹⁰⁴) of hIGFBP-4 and rIGFBP-4 (Fig. 1) and that the glycosylation of IGFBP-4 does not affect its binding to IGFs (Chelius *et al.* 2001). Five different glycosylation isoforms of rIGFBP-4, isolated from rat serum, were recently identified (Chelius *et al.* 2002). All identified oligosaccharides are bi-antennary and differ only in the number of sialic acid terminal residues and/or core modification with fucose. The physiological significance of the glycosylation in IGFBP-4 is unknown.

Proteolysis

Proteolysis is a major regulatory mechanism of IGFBP-4 functions. Each of the six IGFBPs can undergo proteolysis, which results in decreased affinity for IGFs. While some of the IGFBP proteases can use multiple IGFBPs as substrate, there are apparently proteases that are specific for individual IGFBPs (reviews: Maile & Holly 1999, Schneider

et al. 2002). An IGF-dependent IGFBP-4-specific protease was first reported in the media conditioned by both human and sheep dermal fibroblasts (Fowlkes & Freemark 1992), which was then identified as pregnancy-associated plasma protein-A (PAPP-A) (Lawrence *et al.* 1999b). This proteolytic activity has also been detected in the conditioned media from human osteoblasts (X Qin *et al.* 1999), vascular smooth muscle cells (Bayes-Genis *et al.* 2001), granulosa cells (Conover *et al.* 2001), trophoblast and decidualized endometrial stromal cells (Giudice *et al.* 2002), as well as in ovarian follicular fluid (Conover *et al.* 1999) and human pregnancy serum (Byun *et al.* 2001b).

PAPP-A was first isolated from human pregnancy serum (Lin *et al.* 1974) and belongs to the large metzincin family of metalloproteases (Lawrence *et al.* 1999a, Boldt *et al.* 2001). It cleaves IGFBP-4 at a single site, between Met¹³⁵/Lys¹³⁶ in hIGFBP-4 (Fig. 1) (Byun *et al.* 2000, Laursen *et al.* 2002a). IGFBP-4 cleavage by PAPP-A uniquely depends on the presence of IGF (Byun *et al.* 2000, Qin *et al.* 2000, Laursen *et al.* 2001). Recent data suggest that IGFs enhance the proteolysis by binding to IGFBP-4, but not by interaction with PAPP-A (Qin *et al.* 2000, Laursen *et al.* 2001). PAPP-A also cleaves hIGFBP-5 between Ser¹⁴³/Lys¹⁴⁴, which does not require the presence of IGF, but is slightly inhibited by IGF (Laursen *et al.* 2001, Rivera & Fortune 2003). PAPP-A can cleave bovine IGFBP-2 between Gln¹⁶⁵/Met¹⁶⁶ as well, and this proteolytic activity was shown to be dose-dependently enhanced by IGFs (Monget *et al.* 2003). PAPP-A is secreted as a dimer of 400 kDa, but exists in human pregnancy serum as a 500 kDa covalent heterotetrameric 2:2 complex with the proform of eosinophil major basic protein (proMBP), which functions as an inhibitor of the proteolytic activity of PAPP-A (Overgaard *et al.* 2000). The proMBP was also expressed in human fibroblasts after phorbol ester tumor promoter treatment and simian virus 40 transformation, and inhibited the proteolytic activity of IGFBP-4 (Chen *et al.* 2002). IGFBP-3, -5 and -6 can also function as inhibitors of IGFBP-4 proteolysis, probably through the homologous HBD in the C-termini of these IGFBPs (Fowlkes *et al.* 1997). A peptide derived from the HBD of IGFBP-5 (P5) is also able to inhibit the cleavage of IGFBP-2 by PAPP-A, and this inhibition is due to a direct interaction of P5 with PAPP-A rather than with IGFBP-2 (Monget *et al.* 2003). Recent data demonstrate that PAPP-A reversibly binds to the cell surface of several cell types, which does not affect the proteolytic activity of PAPP-A (Laursen *et al.* 2002b). This suggests that adhesion to the cell surface functions to target PAPP-A activity to the vicinity of the IGF receptor, decreasing the probability that released IGF is captured by other IGFBPs before receptor binding. Proteolysis of IGFBP-4 by PAPP-A enhances IGF bioavailability. Its physiological significance will be discussed below.

Table 1 Regulatory mechanisms for IGFBP-4 expression *in vivo*

	Tissue type/species	Effect	Reference
Agent			
Clenbuterol	Soleus muscle/r	s	Awede <i>et al.</i> 2002
EB1089	Prostate/r	s	Nickerson & Huynh 1999
Estrogen	Bone/m	s	Lindberg <i>et al.</i> 2002
	Serum/constitutionally tall girls	s	Rooman <i>et al.</i> 2002
FSH	Ovary/r	s	Putowski <i>et al.</i> 1997
GH	Serum/zinc-deprived rat	s	Ninh <i>et al.</i> 1998
	Serum/postmenopausal woman	s	Kassem <i>et al.</i> 1998
	Serum/bGH transgenic mice	s	Blackburn <i>et al.</i> 1997
GHRA	Serum, liver/m	s	van Neck <i>et al.</i> 2000
GHRP-2	Plasma/b (high feed intake)	s	Lee <i>et al.</i> 2000
	Plasma/b (low feed intake)	n	Lee <i>et al.</i> 2000
hCG	Mammary gland/r	i	Huynh 1998
	Ovary/r	s	Putowski <i>et al.</i> 1997
LPS	Serum/r	s	Soto <i>et al.</i> 1998
Nandrolone	Diaphragm muscle/r	i	Lewis <i>et al.</i> 2002
PMSG	Ovary/r	s	Putowski <i>et al.</i> 1997
T ₃	Kidney, serum/hypothyroid rat	s	Voci <i>et al.</i> 2001
	Liver, serum/hypothyroid rat	s	Demori <i>et al.</i> 1997b
	Uterus/r	s	Bottazzi <i>et al.</i> 1996
T ₄	Liver, mammary gland/r	s	Rosato <i>et al.</i> 2002

EB1089, vitamin D₃ analogue; FSH, follicle-stimulating hormone; GH, growth hormone; GHRA, GH receptor antagonist; GHRP-2, GH-releasing peptide-2; hCG, human chorionic gonadotropin; LPS, lipopolysaccharide; PMSG, pregnant mare's serum gonadotropin; T₃, triiodothyronine; T₄, thyroxine; b, bovine; h, human; m, mouse; r, rat; s, stimulation; i, inhibition; n, no effect.

IGFBP-4 expression *in vivo* and its regulation

IGFBP-4 has been identified in all biological fluids, including serum, follicular fluid, seminal fluid, interstitial fluid and synovial fluid (Rajaram *et al.* 1997). It is the second most abundant IGFBP in adult rat serum after IGFBP-3. Northern blot analysis revealed that IGFBP-4 mRNA is widely expressed in adult rat tissues, including adrenal gland, testis, spleen, heart, liver, lung, kidney, stomach, hypothalamus and brain cortex, with liver being the site of the highest expression (Shimasaki *et al.* 1990). The expression of IGFBP-4 was also examined in rat small intestine (Shoubridge *et al.* 2001), smooth muscle (Smith *et al.* 2001), skeletal muscle (Jennische & Hall 2000), pancreas (Hill *et al.* 1999), uterus and placenta (Cerro & Pintar 1997), mouse spinal cord (Arnold *et al.* 2000), mouse and human thymus (Li *et al.* 1996), human prostate (Thomas *et al.* 2000), bone (Mohan *et al.* 1995a) and ovary (Zhou & Bondy 1993, el Roeiy *et al.* 1994) of several species. In the mouse embryo, IGFBP-4 transcripts were detected as early as 11 days postcoitum (dpc) in different regions, including telencephalon, mesencephalon, snout, tongue and differentiating sclerotomes. After 14 dpc IGFBP-4 mRNA was undetected in the brain, but clearly detectable in lung, liver, kidney, intestine, vertebrae, ribs and incisivi (Cerro *et al.* 1993, Schuller *et al.* 1993). IGFBP-4 protein was localized in telencephalon, mesencephalon, heart, liver, lung, tongue, blood vessels and kidney of 13.5 dpc mouse embryo by immunohistochemi-

cal analysis (van Kleffens *et al.* 1999). These findings suggest that IGFBP-4 expression is developmentally regulated. Moreover, hormones, cytokines and other agents regulate the expression of IGFBP-4 in a tissue-specific manner (see Table 1).

IGFBP-4 expression *in vitro* and its regulation

Consistent with the widespread expression of IGFBP-4 *in vivo*, IGFBP-4 is expressed by various cell types *in vitro*, including fibroblasts, osteoblasts, myoblasts, epithelial cells, endothelial cells, chondrocytes and many kinds of tumor cells. The expression of IGFBP-4 *in vitro* is regulated by a large number of agents in a cell-specific manner. The effects of these agents are summarized in Table 2.

Actions of IGFBP-4

Several lines of evidence suggest that IGFBP-4 functions as a purely inhibitory protein *in vitro* and *in vivo*. These inhibitory actions can be exerted via IGF-dependent and -independent pathways (Fig. 2).

IGF-dependent actions

IGFBP-4 inhibits IGF-induced cell proliferation and differentiation in all cell types studied *in vitro* so far, including bone cells (Schiltz *et al.* 1993, Mohan *et al.* 1995b, Mohan & Baylink 2002), muscle cells (Damon *et al.* 1998a, Ewton

Table 2 Regulatory mechanisms for IGFBP-4 expression *in vitro*

	Cell types/species	Effect	Reference
Agent			
ACTH	Adrenocortical cells/h	n	Fottner <i>et al.</i> 2001
ACTH and IGFs	Adrenocortical cells/h	s	Fottner <i>et al.</i> 2001
Activin	Granulosa cells/r	i	Choi <i>et al.</i> 1997
Androgen	Osteoblasts/h	i	Gori <i>et al.</i> 1999
	Granulosa cells/h	n	Greisen <i>et al.</i> 2002
Angiotensin II	Vascular smooth muscle cells/r	i	Gustafsson <i>et al.</i> 1999b; Anwar <i>et al.</i> 2000
bFGF	Multiple myeloma/h	n	Feliers <i>et al.</i> 1999
	Fetal lung fibroblasts/r	s	Price 1999
cAMP	Multiple myeloma/h	n	Feliers <i>et al.</i> 1999
	Vascular smooth muscle cells/h	s	Hayford <i>et al.</i> 1998
	Osteoblasts/r	s	Chen <i>et al.</i> 1998
	L6 myoblasts/r	s	McCusker & Clemmons 1998
	Articular chondrocytes/h	i	Di Battista <i>et al.</i> 1997
Dexamethasone	Bone marrow stromal cells/r	s	Milne <i>et al.</i> 2001
	Bone marrow stromal cells/h	s	Cheng <i>et al.</i> 1998
	Vascular smooth muscle cells/h	s	Hayford <i>et al.</i> 1998
	TC3 pancreatic beta cells	s	Katz <i>et al.</i> 1997
	Fibroblasts/h,b	i	Conover <i>et al.</i> 1995
EGF	Fetal lung fibroblasts/r	s	Price 1999
Estrogen	MCF-7 breast cancer cells/h	s	C Qin <i>et al.</i> 1999
	SaOS-2 osteoblasts/h	i	Kudo <i>et al.</i> 1996, 1997
	hFOB/ER9 osteoblasts/h	s	Kassem <i>et al.</i> 1996
FSH	Granulosa cells/r	i	Piferrer <i>et al.</i> 1997
GH	TC3 pancreatic beta cells	n	Katz <i>et al.</i> 1997
Glucagon	Ovarian thecal cells/b	n	Chamberlain & Spicer 2001
Glucose	Retinal endothelial cells/h	n	Giannini <i>et al.</i> 2001
IGF-I	Retinal endothelial cells/h	i	Giannini <i>et al.</i> 2001
	Glomerular endothelial cells/h	i	Giannini <i>et al.</i> 1999
	Uterine myometrial cells	s	Huynh 2000
	Smooth muscle cells/h	i	Kuemmerle & Teng 2000
	Vascular smooth muscle cells/r	s	Gustafsson <i>et al.</i> 1999b
	Vascular smooth muscle cells/p	i	Duan & Clemmons 1998
	Vascular smooth muscle cells/h	i	Hayford <i>et al.</i> 1998
	L6 myoblasts/r	s	McCusker & Clemmons 1998
	Fibroblasts/h	i	Conover <i>et al.</i> 1995
	Fibroblasts/b	s	Conover <i>et al.</i> 1995
	SH-SY5Y neuroblastoma cells/h	s	Babajko <i>et al.</i> 1997
IGF-II	Bone marrow stromal cells/h	i	Thomas <i>et al.</i> 1999
	Adrenocortical cells/b	s	Fottner <i>et al.</i> 1999
	TC3 pancreatic beta cells	n	Katz <i>et al.</i> 1997
	Non-small cell lung cancer cells	i	Fottner <i>et al.</i> 1999
	SH-SY5Y neuroblastoma cells/h	s	Babajko <i>et al.</i> 1997
IL-1 β	Ovarian granulosa cells	i	Chamoun <i>et al.</i> 1999
	Articular chondrocytes/h	i	Di Battista <i>et al.</i> 1997
	Fetal lung fibroblast/r	s	Price <i>et al.</i> 2002
IL-6	Hepatocytes/r	s	Fernandez-Celemin & Thissen 2001
	Multiple myeloma/h	n	Feliers <i>et al.</i> 1999
IFN- γ	Multiple myeloma/h	s	Feliers <i>et al.</i> 1999
Insulin	Ovarian thecal cells/b	n	Chamberlain & Spicer 2001
	L6 myoblasts/r	s	McCusker & Clemmons 1998
	TC3 pancreatic beta cells	n	Katz <i>et al.</i> 1997
	SH-SY5Y neuroblastoma cells/h	s	Babajko <i>et al.</i> 1997
	Granulosa cells/h	s	Greisen <i>et al.</i> 2002
Insulin + glucagon	Ovarian thecal cells/b	i	Chamberlain & Spicer 2001
N-myc oncogene	SK-N-SH neuroblastoma cells/h	s	Chambery <i>et al.</i> 1999
OP-1	Osteoblasts/r	i	Yeh <i>et al.</i> 1996

Continued

Table 2 Continued

	Cell types/species	Effect	Reference
Agent PDGF-BB	Fetal lung fibroblasts/r	s	Price 2001
	Multiple myeloma/h	n	Feliers <i>et al.</i> 1999
	Articular chondrocytes/h	s	Di Battista <i>et al.</i> 1997
	SaOS-2 osteoblasts/h	s	Kudo <i>et al.</i> 1996, 1997
PTH	SaOS-2 osteoblasts/h	s	Kudo <i>et al.</i> 1996, 1997; LaTour <i>et al.</i> 1990
	TE85 osteosarcoma cells/h	s	Honda <i>et al.</i> 1996
RA	Sertoli cells/p	s	Bardi <i>et al.</i> 1999
	Neuroblastoma cells/h	i	Chambery <i>et al.</i> 1998
	Osteoblasts/m	s	Glantschnig <i>et al.</i> 1996
	HT-29 colon carcinoma cells	s	Corkins <i>et al.</i> 2002
T ₃	Bone marrow stromal cells/r	s	Milne <i>et al.</i> 2001
	Sertoli cells/p	s	Bardi <i>et al.</i> 1999
	Primary hepatocytes/r	s	Demori <i>et al.</i> 1997a,b
	HepG2 hepatoma cells/h	n	Demori <i>et al.</i> 1997a
TGF- α	Osteoblasts/m	s	Glantschnig <i>et al.</i> 1996
	Granulosa cells/r	s	Piferrer <i>et al.</i> 1997
TGF- β 1	Glomerular endothelial cells/h	s	Giannini <i>et al.</i> 1999
	Multiple myeloma/h	n	Feliers <i>et al.</i> 1999
Thrombin	Stromal-vascular cells/p	s	Richardson <i>et al.</i> 1998
	Vascular smooth muscle cells/r	i	Anwar <i>et al.</i> 2000
TNF- α	Multiple myeloma/h	n	Feliers <i>et al.</i> 1999
	Fetal lung fibroblasts/r	s	Price <i>et al.</i> 2002
D ₃	Bone marrow stromal cells/h	s	Kveiborg <i>et al.</i> 2001
WT-1	Embryonic stem cells/m	s	Wagner <i>et al.</i> 2001

ACTH, adrenocorticotrophic hormone; bFGF, basic fibroblast growth factor; cAMP, cyclic adenosine monophosphate; EGF, epidermal growth factor; FSH, follicle-stimulating hormone; GH, growth hormone; IL, interleukin; IFN, interferon; OP-1, osteogenic protein-1; PDGF-BB, platelet-derived growth factor-BB; PTH, parathyroid hormone; RA, retinoic acid; T₃, triiodothyronine; TGF, transforming growth factor; TNF, tumor necrosis factor; D₃, 1,25-dihydroxyvitamin D₃; WT-1, Wilms tumor-1 protein; b, bovine; h, human; m, mouse; p, porcine; r, rat; s, stimulation; i, inhibition; n, no effect.

et al. 1998, Gustafsson *et al.* 1999a), B104 rat neuroblastoma cells (Cheung *et al.* 1991), HT-29 human colon adenocarcinoma cells (Culouscou & Shoyab 1991) and M12 human prostate cancer cells (Damon *et al.* 1998b). These inhibitory actions of IGFBP-4 have been demonstrated to be IGF-dependent on the basis of the following facts: (i) IGFBP-4 had no effect or lower potency in blocking the biological activity of IGF analogues which have significantly (>100-fold) reduced binding affinity to IGFBP-4 (Mohan *et al.* 1995b); and (ii) IGFBP-4 inhibited the binding of IGF-I to purified IGF-IR *in vitro* (Mohan *et al.* 1995b).

Consistent with the *in vitro* data, IGFBP-4 is also a functional antagonist of IGF actions *in vivo*. For example, transgenic mice overexpressing IGFBP-4 selectively in smooth muscle cells exhibits smooth muscle hypoplasia (Wang *et al.* 1998, Zhang *et al.* 2002), which is in direct contrast to the smooth muscle hypertrophy induced by IGF-I overexpression (Wang *et al.* 1997). Moreover, a protease-resistant IGFBP-4 had more potency (Zhang *et al.* 2002) and IGF-I/IGFBP-4 double transgenic mice showed a reduction in wet weight of selected smooth muscle tissues (Wang *et al.* 1998), suggesting that these inhibitory effects of IGFBP-4 are IGF-I-dependent. In

agreement with the above data, local administration of recombinant IGFBP-4 inhibited IGF-I-induced increases of bone formation in mice (Miyakoshi *et al.* 1999).

In contrast to local IGFBP-4 administration, systemic administration of IGFBP-4 increased bone formation (Miyakoshi *et al.* 1999, 2001). This was the only report regarding a growth-stimulatory effect of IGFBP-4, which was probably by increasing IGF bioavailability via an IGFBP-4 protease-dependent mechanism, since systemic administration of the native IGFBP-4, but not protease-resistant IGFBP-4, increased the levels of serum free IGF-I, serum osteocalcin, serum and skeletal alkaline phosphatase, and IGFBP-4 proteolytic activity in serum (Miyakoshi *et al.* 2001).

IGF-independent actions

In addition to IGF-dependent actions, IGF-independent actions of IGFBP-4 have been suggested, based on the following findings: (i) IGFBP-4 caused a marked inhibition of ceramide-induced apoptosis of Hs578T human breast cancer cells, which lack a functional IGF-IR (Perks *et al.* 1999); (ii) IGFBP-4 inhibited human ovarian steroidogenesis in the presence of either the IGF-IR blocker

Figure 2 IGF-dependent and -independent actions of IGFBP-4 (BP-4). The mitogenic activity of IGFs, mediated by IGF-IR, is inhibited by the binding of IGFBP-4. Proteolysis of IGFBP-4 results in the release of IGFs from the IGF/IGFBP-4 complex and consequently a potentiation of IGF effects. Several potential IGF-independent effects of IGFBP-4 are also documented, but the mechanisms are unknown yet. Refer to the text for further details of these actions.

α IR3 or excess IGFBP-3 to remove the effects of endogenous IGF action (Wright *et al.* 2002); and (iii) endogenous IGFBP-4 inhibited the mitogenic effects of IGF and insulin in HT-29 human colonic adenocarcinoma cells, which could not be compensated for by the addition of an excess of IGF-I and insulin, but by the addition of an antibody against IGFBP-4 (Singh *et al.* 1994). However, unlike IGFBP-3 and -5, a specific receptor for IGFBP-4 has not been identified yet. Further studies are necessary to define the mechanisms of IGF-independent actions of IGFBP-4.

Biological significance of IGFBP-4

Reproductive physiology

The expression pattern of IGFBP-4 and its regulation were extensively studied in human and animal reproductive organs at various reproductive stages. The findings in these studies indicate important roles for IGFBP-4 in reproduction.

Pregnancy IGFs, as mitogenic peptides, are important for fetal and placental growth during pregnancy (Han & Carter 2000). In the human placenta, IGFs regulate syncytiotrophoblast steroidogenesis (Nestler 1987, 1990), glucose and amino acid transport in the villi (Kniss *et al.*

1994) and the invasion of the extravillous trophoblast into the maternal decidua (Han *et al.* 1996, McKinnon *et al.* 2001). IGFBPs are expressed by the maternal decidua and may regulate IGF actions during pregnancy (Han *et al.* 1996). In this context IGFBP-4 is particularly interesting for several reasons: (i) IGFBP-4 is the second most abundant IGFBP in the placental bed (Han *et al.* 1996); (ii) PAPP-A, an IGF-dependent IGFBP-4 protease, is secreted by human trophoblast cells and decidualized endometrial stroma (Giudice *et al.* 2002, Sun *et al.* 2002) and is markedly increased in the maternal circulation as pregnancy progresses (Byun *et al.* 2000); and (iii) in human pregnancy serum the majority of PAPP-A (>99%) is found as a PAPP-A/proMBP complex (Oxvig *et al.* 1993). During pregnancy, rapid placental development and fetal growth obviously increases the need for growth-promoting factors such as IGFs. The mitogenic activity of IGFs at the local cellular level depends on the concentration of free IGFs that are able to interact with their receptors. The increased IGFBP-4 proteolytic activity resulting from uncomplexed PAPP-A may be required locally to increase the concentration of free IGFs for placental development and therefore for fetal growth during pregnancy, whereas the PAPP-A activity in maternal circulation is inhibited by complex formation with proMBP. The absence of PAPP-A expression in the placenta of pregnant patients with Cornelia de Lange syndrome, a condition involving incomplete fetal development and subsequent deformities

(Westergaard *et al.* 1983), provides direct evidence for a role of PAPP-A in pregnancy. However, the roles of this complex system of enzyme (PAPP-A), substrate (IGFBP-4), inhibitor (proMBP) and cofactor (IGF-II) in the placenta and maternal circulation during human pregnancy deserve further investigation.

Different from human, recent data showed that the IGFBP-4 proteolytic activity in murine serum is not increased during pregnancy, eventually due to the lower level of PAPP-A expression in the placenta (Qin *et al.* 2002, Soe *et al.* 2002). The significance of this difference between species is unknown, but this difference must be taken into consideration when the mouse is used as a model organism for the study of PAPP-A function.

Ovarian physiology IGFs are produced by ovarian granulosa and theca cells, and mediate gonadotropin actions on ovarian cellular steroidogenesis and growth (Poretsky *et al.* 1999). Regulation of IGF actions within the ovarian follicle is particularly important in the processes of ovarian follicle development and follicle atresia (Monget *et al.* 2002). As a potent inhibitor of IGF actions, IGFBP-4 appears to be particularly important in ovarian physiology (Iwashita *et al.* 1996, Poretsky *et al.* 1999). High levels of IGFBP-4 are present in small androgen-dominant follicles (Zhou & Bondy 1993, el Roeiy *et al.* 1994) and in follicular fluid from androgen-dominant follicles (FF_a) that are growth-arrested or atretic (Cataldo & Giudice 1992, San Roman & Magoffin 1993). In contrast, IGFBP-4 is undetected by ligand blot analysis in follicular fluid from estrogen-dominant growing follicles (FF_e). IGFBP-4 inhibits human ovarian steroidogenesis *in vivo* (Mason *et al.* 1998, Wright *et al.* 2002), and it has been suggested that IGFBP-4 inhibits follicle development by inhibiting IGF actions in the ovary, and conversely, the loss of this inhibitory factor allows for increased bioavailable IGFs, which coincides with selection of the dominant follicle (Poretsky *et al.* 1999). The complexity of this process has become even more apparent with the finding of the IGFBP-4-specific protease PAPP-A activity in FF_e, but not in FF_a (Chandrasekher *et al.* 1995). PAPP-A is expressed in human and mouse ovaries, being restricted to healthy granulosa cells and granulosa-lutein cells (Hourvitz *et al.* 2000, 2002). This restricted expression pattern and its co-expression with aromatase and luteinizing hormone receptor in granulosa cells from preovulatory follicles suggests that PAPP-A could be considered as a functional marker of follicular development (Mazerbourg *et al.* 2001). During terminal development of follicles to the preovulatory stage, the degradation of IGFBP-4 by PAPP-A in the ovary may increase IGF bioavailability that further stimulates granulosa cell proliferation and steroidogenesis, and then actively participates in the selection of dominant follicles *in vivo*. In contrast, in atretic follicles, IGFBP-4 degradation is inhibited by locally increased IGFBP-2 and -5 that contain an HBD in their C-terminal

regions (Mazerbourg *et al.* 2000). The IGFBP-4 protease activity has been reported in human, ovine, bovine, porcine, equine (Mazerbourg *et al.* 2000, 2001) and murine ovaries (Hourvitz *et al.* 2002), suggesting a well-conserved process for this protease in ovarian function.

Bone formation

IGFs are the most abundant growth factors stored in the bone and regulate the proliferation and differentiation of bone cells (Bautista *et al.* 1990). As in other tissues, the local activity of IGFs in bone is modulated by IGFBPs. IGFBP-4 is one of the major IGFBPs produced by bone cells (Mohan *et al.* 1995b) and has been proposed to function as an important regulator of bone formation. Evidence was provided by the following findings: (i) IGFBP-4 inhibited both basal and IGF-induced cell proliferation of MC3T3-E1 mouse osteoblasts and untransformed normal human bone cells (Mohan *et al.* 1995b) and the growth of embryonic chicken pelvic cartilage *in vitro* (Schiltz *et al.* 1993); (ii) a single local administration of IGFBP-4 inhibited IGF-I-induced increases in bone formation, whereas systemic administration of IGFBP-4 alone increased serum and skeletal levels of bone formation markers (osteocalcin and alkaline phosphatase) in mice (Miyakoshi *et al.* 1999, 2001). The latter stimulatory effect resulted from an increase of IGF bioavailability in the circulation via an IGFBP-4 protease-dependent mechanism (Miyakoshi *et al.* 2001); (iii) the serum level of IGFBP-4 was shown to increase with aging and to correlate positively with serum PTH levels (Rajaram *et al.* 1997, Karasik *et al.* 2002). PTH upregulated IGFBP-4 expression in human osteoblasts *in vitro*, and serum IGFBP-4 levels were found to be increased during oral 1,25-dihydroxyvitamin D₃ therapy in psoriasis patients. These findings suggest that during calcium deficiency, the increase in serum PTH and 1,25-dihydroxyvitamin D₃ may not only stimulate bone resorption, but also inhibit bone formation by stimulating IGFBP-4 production in bone cells (Mohan *et al.* 1995a, Rajaram *et al.* 1997, Karasik *et al.* 2002); and (iv) sera from patients with chronic renal failure (CRF) usually contain high levels of IGFBP-4, which may contribute to decreased bone formation in renal osteodystrophy (Van Doorn *et al.* 2001).

Renal pathophysiology

IGFBP-4 is abundantly expressed in the kidney. A site-specific expression pattern of IGFBP-4 during nephrogenesis was described in the human (Matsell *et al.* 1994), rat (G J Price *et al.* 1995) and mouse (Lindenbergh-Kortleve *et al.* 1997), suggesting specific roles for IGFBP-4 in renal development and physiology. In addition, changes in IGFBP-4 abundance may be associated with pathological processes of the kidney. Upregulation of IGFBP-4 levels in serum correlated with the degree of renal

dysfunction and growth retardation of children with CRF (Uliniski *et al.* 2000, Van Doorn *et al.* 2001), while downregulation of renal IGFBP-4 expression was reported in growth hormone-induced rat hypersomatotrophy (Hise *et al.* 2001).

IGFBP-4 and cancer

IGFBP-4 is expressed in a range of cells of tumor origin, such as lung adenocarcinoma (W A Price *et al.* 1995), non-small-cell lung cancer (Noll *et al.* 1996), breast cancer (C Qin *et al.* 1999), colon carcinoma (Michell *et al.* 1997), follicular thyroid carcinoma (Bachrach *et al.* 1995), gastric cancer (Yi *et al.* 2001), glioma (Bradshaw *et al.* 1999), hepatoma (Scharf *et al.* 1998), myeloma (Feliars *et al.* 1999), neuroblastoma (Cheung *et al.* 1991, Babajko & Binoux 1996), osteosarcoma (Mohan *et al.* 1995b) and prostate cancer (Srinivasan *et al.* 1996, Damon *et al.* 1998b, Drivdahl *et al.* 2001).

In vitro and *in vivo* studies suggest that IGFBP-4 plays an important role in the growth regulation of a variety of tumors, possibly by inhibiting autocrine IGF actions or by as yet unknown IGF-independent mechanisms. Notably, in Caco-2 human colon carcinoma cells expression of IGFBP-4 mRNA was correlated with cell differentiation, indicating growth inhibitory effects in that cellular system (Hoeflich *et al.* 1996). Proliferation, anchorage-independent growth and tumor development in athymic nude mice were inhibited by overexpression of IGFBP-4 in M12 prostate cancer cells. Apoptosis was increased in the IGFBP-4-overexpressing cells, probably due to sequestering IGF ligands (Damon *et al.* 1998b). Blocking of IGFBP-4 with antibodies enhanced both basal and IGF-stimulated growth of HT-29 human colonic carcinoma cells in both an IGF-dependent and an IGF-independent manner (Singh *et al.* 1994). Recombinant IGFBP-4 caused marked inhibition of ceramide-induced apoptosis of Hs578T human breast cancer cells via an IGF-independent pathway (Perks *et al.* 1999).

Conclusions

IGFBP-4 is the smallest of the six high-affinity IGFBPs. It exists in both non-glycosylated and glycosylated forms in all biological fluids. IGFBP-4 is expressed by a large range of cell types and tissues, and its expression is affected by different mechanisms in a cell type-specific manner. IGFBP-4 binds IGF-I and IGF-II with similar affinities and inhibits their actions in almost all *in vitro* and *in vivo* conditions. IGF-independent actions have also been reported. Proteolysis is a major regulatory mechanism for IGFBP-4. An IGF-dependent IGFBP-4-specific protease has been identified, which plays important roles in regulating IGFBP-4 actions in reproductive physiology. In addition, IGFBP-4 is an important regulator for bone

formation and renal pathophysiology. Further studies are necessary to address the following questions: (i) Can IGFBP-4 enhance IGF actions? (ii) What is the mechanism underlying the IGF-independent actions of IGFBP-4? (iii) What is the subcellular distribution of IGFBP-4? (iv) What are the specific roles for IGFBP-4 *in vivo*? Biochemical, molecular biological and transgenic approaches will widen our understanding of the nature of this binding protein.

Acknowledgement

R Zhou is a recipient of a grant from the German Academic Exchange Service (DAAD).

References

- Allander SV, Bajalica S, Larsson C, Luthman H, Powell DR, Stern I, Weber G, Zazzi H & Ehrenborg E 1993 Structure and chromosomal localization of human insulin-like growth factor-binding protein genes. *Growth Regulation* **3** 3–5.
- Andress DL 1995 Heparin modulates the binding of insulin-like growth factor (IGF) binding protein-5 to a membrane protein in osteoblastic cells. *Journal of Biological Chemistry* **270** 28289–28296.
- Andress DL 1998 Insulin-like growth factor-binding protein-5 (IGFBP-5) stimulates phosphorylation of the IGFBP-5 receptor. *American Journal of Physiology* **274** E744–E750.
- Anwar A, Zahid AA, Phillips L & Delafontaine P 2000 Insulin-like growth factor binding protein-4 expression is decreased by angiotensin II and thrombin in rat aortic vascular smooth muscle cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* **20** 370–376.
- Arai T, Busby W Jr & Clemmons DR 1996 Binding of insulin-like growth factor (IGF) I or II to IGF-binding protein-2 enables it to bind to heparin and extracellular matrix. *Endocrinology* **137** 4571–4575.
- Arnold PM, Ma JY, Citron BA, Zoubine MN & Festoff BW 2000 Selective developmental regulation of gene expression for insulin-like growth factor-binding proteins in mouse spinal cord. *Spine* **25** 1765–1770.
- Awede BL, Thissen JP & Lebacqz J 2002 Role of IGF-I and IGFBPs in the changes of mass and phenotype induced in rat soleus muscle by clenbuterol. *American Journal of Physiology* **282** E31–E37.
- Babajko S & Binoux M 1996 Modulation by retinoic acid of insulin-like growth factor (IGF) and IGF binding protein expression in human SK-N-SH neuroblastoma cells. *European Journal of Endocrinology* **134** 474–480.
- Babajko S, Leneuve P, Loret C & Binoux M 1997 IGF-binding protein-6 is involved in growth inhibition in SH-SY5Y human neuroblastoma cells: its production is both IGF- and cell density-dependent. *Journal of Endocrinology* **152** 221–227.
- Bachrach LK, Nanto-Salonen K, Tapanainen P, Rosenfeld RG & Gargosky SE 1995 Insulin-like growth factor binding protein production in human follicular thyroid carcinoma cells. *Growth Regulation* **5** 109–118.
- Bar RS, Clemmons DR, Boes M, Busby WH, Booth BA, Dake BL & Sandra A 1990 Transcapillary permeability and subendothelial distribution of endothelial and amniotic fluid insulin-like growth factor binding proteins in the rat heart. *Endocrinology* **127** 1078–1086.
- Bardi G, Bottazzi C, Demori I & Palmero S 1999 Thyroid hormone and retinoic acid induce the synthesis of insulin-like growth factor-binding protein-4 in prepubertal pig sertoli cells. *European Journal of Endocrinology* **141** 637–643.

- Batzer MA & Deininger PL 2002 Alu repeats and human genomic diversity. *Nature Reviews. Genetics* **3** 370–379.
- Bautista CM, Mohan S & Baylink DJ 1990 Insulin-like growth factors I and II are present in the skeletal tissues of ten vertebrates. *Metabolism* **39** 96–100.
- Bayes-Genis A, Schwartz RS, Lewis DA, Overgaard MT, Christiansen M, Oxvig C, Ashai K, Holmes DR Jr & Conover CA 2001 Insulin-like growth factor binding protein-4 protease produced by smooth muscle cells increases in the coronary artery after angioplasty. *Arteriosclerosis, Thrombosis, and Vascular Biology* **21** 335–341.
- Blackburn A, Dressendorfer RA, Blum WF, Erhard M, Brem G, Strasburger CJ & Wolf E 1997 Interactions of insulin-like growth factor (IGF)-II and growth hormone *in vivo*: circulating levels of IGF-I and IGF-binding proteins in transgenic mice. *European Journal of Endocrinology* **137** 701–708.
- Boes M, Booth BA, Sandra A, Dake BL, Bergold A & Bar RS 1992 Insulin-like growth factor binding protein (IGFBP)4 accounts for the connective tissue distribution of endothelial cell IGFBPs perfused through the isolated heart. *Endocrinology* **131** 327–330.
- Boldt HB, Overgaard MT, Laursen LS, Weyer K, Sottrup-Jensen L & Oxvig C 2001 Mutational analysis of the proteolytic domain of pregnancy-associated plasma protein-A (PAPP-A): classification as a metzincin. *Biochemical Journal* **358** 359–367.
- Booth BA, Boes M, Andress DL, Dake BL, Kiefer MC, Maack C, Linhardt RJ, Bar K, Caldwell EE, Weiler J *et al.* 1995 IGFBP-3 and IGFBP-5 association with endothelial cells: role of C-terminal heparin binding domain. *Growth Regulation* **5** 1–17.
- Bottazzi C, Demori I, Leone M & Fugassa E 1996 Thyroid hormone affects rat uterine expression of IGF-I and IGFBP-4. *Bollettino della Società Italiana di Biologia Sperimentale* **72** 133–138.
- Bradshaw SL, D'Ercole AJ & Han VK 1999 Overexpression of insulin-like growth factor-binding protein-2 in C6 glioma cells results in conditional alteration of cellular growth. *Endocrinology* **140** 575–584.
- Braulke T 1999 Type-2 IGF receptor: a multi-ligand binding protein. *Hormone and Metabolism Research* **31** 242–246.
- Butler AA & LeRoith D 2001 Minireview: tissue-specific versus generalized gene targeting of the IGF1 and IGF1R genes and their roles in insulin-like growth factor physiology. *Endocrinology* **142** 1685–1688.
- Byun D, Mohan S, Kim C, Suh K, Yoo M, Lee H, Baylink DJ & Qin X 2000 Studies on human pregnancy-induced insulin-like growth factor (IGF)-binding protein-4 proteases in serum: determination of IGF-II dependency and localization of cleavage site. *Journal of Clinical Endocrinology and Metabolism* **85** 373–381.
- Byun D, Mohan S, Baylink DJ & Qin X 2001a Localization of the IGF binding domain and evaluation of the role of cysteine residues in IGF binding in IGF binding protein-4. *Journal of Endocrinology* **169** 135–143.
- Byun D, Mohan S, Yoo M, Sexton C, Baylink DJ & Qin X 2001b Pregnancy-associated plasma protein-A accounts for the insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) proteolytic activity in human pregnancy serum and enhances the mitogenic activity of IGF by degrading IGFBP-4 *in vitro*. *Journal of Clinical Endocrinology and Metabolism* **86** 847–854.
- Calabretta B, Robberson DL, Barrera-Saldana HA, Lambrou TP & Saunders GF 1982 Genome instability in a region of human DNA enriched in Alu repeat sequences. *Nature* **296** 219–225.
- Carr JM, Grant PA, Francis GL, Owens JA, Wallace JC & Walton PE 1994 Isolation and characterization of ovine IGFBP-4: protein purification and cDNA sequence. *Journal of Molecular Endocrinology* **13** 219–236.
- Cataldo NA & Giudice LC 1992 Insulin-like growth factor binding protein profiles in human ovarian follicular fluid correlate with follicular functional status. *Journal of Clinical Endocrinology and Metabolism* **74** 821–829.
- Ceda GP, Fielder PJ, Henzel WJ, Louie A, Donovan SM, Hoffman AR & Rosenfeld RG 1991 Differential effects of insulin-like growth factor (IGF)-I and IGF-II on the expression of IGF binding proteins (IGFBPs) in a rat neuroblastoma cell line: isolation and characterization of two forms of IGFBP-4. *Endocrinology* **128** 2815–2824.
- Cerro JA & Pintar JE 1997 Insulin-like growth factor binding protein gene expression in the pregnant rat uterus and placenta. *Developmental Biology* **184** 278–295.
- Cerro JA, Grewal A, Wood TL & Pintar JE 1993 Tissue-specific expression of the insulin-like growth factor binding protein (IGFBP) mRNAs in mouse and rat development. *Regulatory Peptides* **48** 189–198.
- Chamberlain CS & Spicer LJ 2001 Hormonal control of ovarian cell production of insulin-like growth factor binding proteins. *Molecular and Cellular Endocrinology* **182** 69–81.
- Chambery D, de Galle B & Babajko S 1998 Retinoic acid stimulates IGF binding protein (IGFBP)-6 and depresses IGFBP-2 and IGFBP-4 in SK-N-SH human neuroblastoma cells. *Journal of Endocrinology* **159** 227–232.
- Chambery D, Mohseni-Zadeh S, de Galle B & Babajko S 1999 N-myc regulation of type I insulin-like growth factor receptor in a human neuroblastoma cell line. *Cancer Research* **59** 2898–2902.
- Chamoun D, DeMoura MD, Levitas E, Resnick CE, Gargosky SE, Rosenfeld RG, Matsumoto T & Adashi EY 1999 Transcriptional and posttranscriptional regulation of intraovarian insulin-like growth factor-binding proteins by interleukin-1 beta (IL-1 beta): evidence for IL-1 beta as an antiatretic principle. *Endocrinology* **140** 3488–3495.
- Chandrasekher YA, Van Dessel HJ, Fauser BC & Giudice LC 1995 Estrogen- but not androgen-dominant human ovarian follicular fluid contains an insulin-like growth factor binding protein-4 protease. *Journal of Clinical Endocrinology and Metabolism* **80** 2734–2739.
- Chelius D, Baldwin MA, Lu X & Spencer EM 2001 Expression, purification and characterization of the structure and disulfide linkages of insulin-like growth factor binding protein-4. *Journal of Endocrinology* **168** 283–296.
- Chelius D, Wu SL & Bondarenko PV 2002 Identification of N-linked oligosaccharides of rat insulin-like growth factor binding protein-4. *Growth Hormone and IGF Research* **12** 169–177.
- Chen BK, Overgaard MT, Bale LK, Resch ZT, Christiansen M, Oxvig C & Conover CA 2002 Molecular regulation of the IGF-binding protein-4 protease system in human fibroblasts: identification of a novel inducible inhibitor. *Endocrinology* **143** 1199–1205.
- Chen Y, Shu H, Ji C, Casaghi S, Kim K, Gundberg CM, Centrella M & McCarthy TL 1998 Insulin-like growth factor binding proteins localize to discrete cell culture compartments in periosteal and osteoblast cultures from fetal rat bone. *Journal of Cellular Biochemistry* **71** 351–362.
- Cheng SL, Zhang SF, Mohan S, Lecanda F, Fausto A, Hunt AH, Canalis E & Avioli LV 1998 Regulation of insulin-like growth factors I and II and their binding proteins in human bone marrow stromal cells by dexamethasone. *Journal of Cellular Biochemistry* **71** 449–458.
- Cheung PT, Smith EP, Shimasaki S, Ling N & Chernauek SD 1991 Characterization of an insulin-like growth factor binding protein (IGFBP-4) produced by the B104 rat neuronal cell line: chemical and biological properties and differential synthesis by sublines. *Endocrinology* **129** 1006–1015.
- Choi D, Rohan RM, Rosenfeld RG, Matsumoto T, Gargosky SE & Adashi EY 1997 Activin-attenuated expression of transcripts encoding granulosa cell-derived insulin-like growth factor binding proteins 4 and 5 in the rat: a putative antiatretic effect. *Biology of Reproduction* **56** 508–515.
- Clemmons DR 1997 Insulin-like growth factor binding proteins and their role in controlling IGF actions. *Cytokine and Growth Factor Reviews* **8** 45–62.

- Cohick WS & Clemmons DR 1993 The insulin-like growth factors. *Annual Review of Physiology* **55** 131–153.
- Conover CA, Clarkson JT & Bale LK 1995 Effect of glucocorticoid on insulin-like growth factor (IGF) regulation of IGF-binding protein expression in fibroblasts. *Endocrinology* **136** 1403–1410.
- Conover CA, Oxvig C, Overgaard MT, Christiansen M & Giudice LC 1999 Evidence that the insulin-like growth factor binding protein-4 protease in human ovarian follicular fluid is pregnancy associated plasma protein-A. *Journal of Clinical Endocrinology and Metabolism* **84** 4742–4745.
- Conover CA, Faessen GF, Ilg KE, Chandrasekhar YA, Christiansen M, Overgaard MT, Oxvig C & Giudice LC 2001 Pregnancy-associated plasma protein-A is the insulin-like growth factor binding protein-4 protease secreted by human ovarian granulosa cells and is a marker of dominant follicle selection and the corpus luteum. *Endocrinology* **142** 2155.
- Corkins MR, McQuade J, Schaffer BS & MacDonald RG 2002 Insulin-like growth factor binding protein-4 expression is dependent on the carbohydrate in the media in HT-29 cells. *Growth Hormone and IGF Research* **12** 184–192.
- Cubbage ML, Suwanichkul A & Powell DR 1990 Insulin-like growth factor binding protein-3. Organization of the human chromosomal gene and demonstration of promoter activity. *Journal of Biological Chemistry* **265** 12642–12649.
- Culouscou JM & Shoyab M 1991 Purification of a colon cancer cell growth inhibitor and its identification as an insulin-like growth factor binding protein. *Cancer Research* **51** 2813–2819.
- Dai B, Widen SG, Mifflin R & Singh P 1997 Cloning of the functional promoter for human insulin-like growth factor binding protein-4 gene: endogenous regulation. *Endocrinology* **138** 332–343.
- Damon SE, Haugk KL, Birnbaum RS & Quinn LS 1998a Retrovirally mediated overexpression of insulin-like growth factor binding protein 4: evidence that insulin-like growth factor is required for skeletal muscle differentiation. *Journal of Cellular Physiology* **175** 109–120.
- Damon SE, Maddison L, Ware JL & Plymate SR 1998b Overexpression of an inhibitory insulin-like growth factor binding protein (IGFBP), IGFBP-4, delays onset of prostate tumor formation. *Endocrinology* **139** 3456–3464.
- De Meyts P & Whittaker J 2002 Structural biology of insulin and IGF1 receptors: implications for drug design. *Nature Reviews. Drug Discovery* **1** 769–783.
- Demori I, Bottazzi C, Arzani D, Voci A & Fugassa E 1997a Regulation of IGFBP-1 and -4 expression by triiodothyronine (T3) in cultured hepatocytes is cell- and gene-specific. *Bollettino della Società Italiana di Biologia Sperimentale* **73** 47–53.
- Demori I, Bottazzi C, Voci A, Gallo G, Scharf JG & Fugassa E 1997b Tri-iodothyronine increases insulin-like growth factor binding protein-4 expression in rat hepatocytes. *Journal of Endocrinology* **154** 155–165.
- Di Battista JA, Dore S, Morin N, He Y, Pelletier JP & Martel-Pelletier J 1997 Prostaglandin E2 stimulates insulin-like growth factor binding protein-4 expression and synthesis in cultured human articular chondrocytes: possible mediation by Ca(++)-calmodulin regulated processes. *Journal of Cellular Biochemistry* **65** 408–419.
- Drivdahl RH, Sprenger C, Trimm K & Plymate SR 2001 Inhibition of growth and increased expression of insulin-like growth factor-binding protein-3 (IGFBP-3) and -6 in prostate cancer cells stably transfected with antisense IGFBP-4 complementary deoxyribonucleic acid. *Endocrinology* **142** 1990–1998.
- Duan C 2002 Specifying the cellular responses to IGF signals: roles of IGF-binding proteins. *Journal of Endocrinology* **175** 41–54.
- Duan C & Clemmons DR 1998 Differential expression and biological effects of insulin-like growth factor-binding protein-4 and -5 in vascular smooth muscle cells. *Journal of Biological Chemistry* **273** 16836–16842.
- el Roeiy A, Chen X, Roberts VJ, Shimasakai S, Ling N, LeRoith D, Roberts CT Jr & Yen SS 1994 Expression of the genes encoding the insulin-like growth factors (IGF-I and II), the IGF and insulin receptors, and IGF-binding proteins-1–6 and the localization of their gene products in normal and polycystic ovary syndrome ovaries. *Journal of Clinical Endocrinology and Metabolism* **78** 1488–1496.
- Ewton DZ, Coolican SA, Mohan S, Chernausk SD & Florini JR 1998 Modulation of insulin-like growth factor actions in L6A1 myoblasts by insulin-like growth factor binding protein (IGFBP)-4 and IGFBP-5: a dual role for IGFBP-5. *Journal of Cellular Physiology* **177** 47–57.
- Feliers D, Woodruff K & Abboud S 1999 Potential role of insulin-like growth factor binding protein-4 in the uncoupling of bone turnover in multiple myeloma. *British Journal of Haematology* **104** 715–722.
- Fernandez-Celemin L & Thissen JP 2001 Interleukin-6 stimulates hepatic insulin-like growth factor binding protein-4 messenger ribonucleic acid and protein. *Endocrinology* **142** 241–248.
- Forbes BE, Turner D, Hodge SJ, McNeil KA, Forsberg G & Wallace JC 1998 Localization of an insulin-like growth factor (IGF) binding site of bovine IGF binding protein-2 using disulfide mapping and deletion mutation analysis of the C-terminal domain. *Journal of Biological Chemistry* **273** 4647–4652.
- Fottner C, Engelhardt D & Weber MM 1999 Characterization of insulin-like growth factor binding proteins (IGFBPs) secreted by bovine adrenocortical cells in primary culture: regulation by insulin-like growth factors (IGFs) and adrenocorticotropin (ACTH). *Hormone and Metabolism Research* **31** 203–208.
- Fottner C, Engelhardt D, Elmlinger MW & Weber MM 2001 Identification and characterization of insulin-like growth factor (IGF)-binding protein expression and secretion by adult human adrenocortical cells: differential regulation by IGFs and adrenocorticotropin. *Journal of Endocrinology* **168** 465–474.
- Fowlkes J & Freemark M 1992 Evidence for a novel insulin-like growth factor (IGF)-dependent protease regulating IGF-binding protein-4 in dermal fibroblasts. *Endocrinology* **131** 2071–2076.
- Fowlkes JL, Thraill KM, George-Nascimento C, Rosenberg CK & Serra DM 1997 Heparin-binding, highly basic regions within the thyroglobulin type-1 repeat of insulin-like growth factor (IGF)-binding proteins (IGFBPs) -3, -5, and -6 inhibit IGFBP-4 degradation. *Endocrinology* **138** 2280–2285.
- Gao L, Ling N & Shimasaki S 1993 Structure of the rat insulin-like growth factor binding protein-4 gene. *Biochemical and Biophysical Research Communications* **190** 1053–1059.
- Giannini S, Cresci B, Pala L, Ciucci A, Manuelli C, Fujita-Yamaguchi Y, Cappugi P & Rotella CM 1999 Human glomerular endothelial cells IGFBPs are regulated by IGF-I and TGF-beta1. *Molecular and Cellular Endocrinology* **154** 123–136.
- Giannini S, Cresci B, Pala L, Ciucci A, Franchini A, Manuelli C, Fujita-Yamaguchi Y, Cappugi P, Zonfrati R & Rotella CM 2001 IGFBPs modulate IGF-I- and high glucose-controlled growth of human retinal endothelial cells. *Journal of Endocrinology* **171** 273–284.
- Giudice LC, Conover CA, Bale L, Faessen GH, Ilg K, Sun I, Imani B, Suen LF, Irwin JC, Christiansen M *et al.* 2002 Identification and regulation of the IGFBP-4 protease and its physiological inhibitor in human trophoblasts and endometrial stroma: evidence for paracrine regulation of IGF-II bioavailability in the placental bed during human implantation. *Journal of Clinical Endocrinology and Metabolism* **87** 2359–2366.
- Glantschnig H, Varga F & Klaushofer K 1996 Thyroid hormone and retinoic acid induce the synthesis of insulin-like growth factor-binding protein-4 in mouse osteoblastic cells. *Endocrinology* **137** 281–286.
- Glantschnig H, Varga F, Luegmayer E & Klaushofer K 1998 Characterization of the mouse insulin-like growth factor binding protein 4 gene regulatory region and expression studies. *DNA and Cell Biology* **17** 51–60.

- Gori F, Hofbauer LC, Conover CA & Khosla S 1999 Effects of androgens on the insulin-like growth factor system in an androgen-responsive human osteoblastic cell line. *Endocrinology* **140** 5579–5586.
- Greisen S, Flyvbjerg A, Ledet T & Ovesen P 2002 Regulation of insulin-like growth factor binding protein secretion by human granulosa luteal cells in a polycystic ovary-like environment. *Fertility and Sterility* **78** 162–168.
- Gustafsson T, Andersson P & Arnqvist HJ 1999a Different inhibitory actions of IGFBP-1, -2 and -4 on IGF-I effects in vascular smooth muscle cells. *Journal of Endocrinology* **161** 245–253.
- Gustafsson T, Andersson P, Chen Y, Magnusson JO & Arnqvist HJ 1999b Interaction of angiotensin II and the insulin-like growth factor system in vascular smooth muscle cells. *American Journal of Physiology* **277** H499–H507.
- Han VK & Carter AM 2000 Spatial and temporal patterns of expression of messenger RNA for insulin-like growth factors and their binding proteins in the placenta of man and laboratory animals. *Placenta* **21** 289–305.
- Han VK, Bassett N, Walton J & Challis JR 1996 The expression of insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) genes in the human placenta and membranes: evidence for IGF-IGFBP interactions at the feto-maternal interface. *Journal of Clinical Endocrinology and Metabolism* **81** 2680–2693.
- Hashimoto R, Ono M, Fujiwara H, Higashihashi N, Yoshida M, Enjoh-Kimura T & Sakano K 1997 Binding sites and binding properties of binary and ternary complexes of insulin-like growth factor-II (IGF-II), IGF-binding protein-3, and acid-labile subunit. *Journal of Biological Chemistry* **272** 27936–27942.
- Hassan AB 2003 Keys to the hidden treasures of the mannose 6-phosphate/insulin-like growth factor 2 receptor. *American Journal of Pathology* **162** 3–6.
- Hayford K, Boes M, Dake BL & Bar RS 1998 Regulations of IGF binding proteins in human aorta vascular smooth muscle cells by cAMP, dexamethasone and IGF-I. *Growth Hormone and IGF Research* **8** 369–375.
- Hill DJ, Hogg J, Petrik J, Arany E & Han VK 1999 Cellular distribution and ontogeny of insulin-like growth factors (IGFs) and IGF binding protein messenger RNAs and peptides in developing rat pancreas. *Journal of Endocrinology* **160** 305–317.
- Hise MK, Salmanullah M, Tannenbaum GS & Rohan RM 2001 mRNA expression of the IGF system in the kidney of the hypersomatotropic rat. *Nephron* **88** 360–367.
- Hoefflich A, Yang Y, Huber S, Rascher W, Koepf G, Blum WF, Heinz-Erian P, Kolb HJ & Kiess W 1996 Expression of IGFBP-2, -3, and -4 mRNA during differentiation of Caco-2 colon epithelial cells. *American Journal of Physiology* **271** E922–E931.
- Hoefflich A, Reisinger R, Vargas GA, Elmlinger MW, Schuett B, Jehle PM, Renner-Muller I, Lahm H, Russo VC & Wolf E 2002 Mutation of the RGD sequence does not affect plasma membrane association and growth inhibitory effects of elevated IGFBP-2 *in vivo*. *FEBS Letters* **523** 63–67.
- Honda Y, Landale EC, Strong DD, Baylink DJ & Mohan S 1996 Recombinant synthesis of insulin-like growth factor-binding protein-4 (IGFBP-4): development, validation, and application of a radioimmunoassay for IGFBP-4 in human serum and other biological fluids. *Journal of Clinical Endocrinology and Metabolism* **81** 1389–1396.
- Houck CM, Rinehart FP & Schmid CW 1979 A ubiquitous family of repeated DNA sequences in the human genome. *Journal of Molecular Biology* **132** 289–306.
- Hourvitz A, Widger AE, Filho FL, Chang RJ, Adashi EY & Erickson GF 2000 Pregnancy-associated plasma protein-A gene expression in human ovaries is restricted to healthy follicles and corpora lutea. *Journal of Clinical Endocrinology and Metabolism* **85** 4916–4920.
- Hourvitz A, Kuwahara A, Hennebold JD, Tavares AB, Negishi H, Lee TH, Erickson GF & Adashi EY 2002 The regulated expression of the pregnancy-associated plasma protein-A in the rodent ovary: a proposed role in the development of dominant follicles and of corpora lutea. *Endocrinology* **143** 1833–1844.
- Huynh H 1998 *In vivo* regulation of the insulin-like growth factor system of mitogens by human chorionic gonadotropin. *International Journal of Oncology* **13** 571–575.
- Huynh H 2000 Post-transcriptional and post-translational regulation of insulin-like growth factor binding protein-3 and -4 by insulin-like growth factor-I in uterine myometrial cells. *Growth Hormone and IGF Research* **10** 20–27.
- Hwa V, Oh Y & Rosenfeld RG 1999 The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocrine Reviews* **20** 761–787.
- Iwashita M, Kudo Y, Yoshimura Y, Adachi T, Katayama E & Takeda Y 1996 Physiological role of insulin-like-growth-factor-binding protein-4 in human folliculogenesis. *Hormone Research* **46** (Suppl 1) 31–36.
- Jennische E & Hall CM 2000 Expression and localisation of IGF-binding protein mRNAs in regenerating rat skeletal muscle. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* **108** 747–755.
- Jones JI & Clemmons DR 1995 Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews* **16** 3–34.
- Jones JI, Gockerman A, Busby WH Jr, Wright G & Clemmons DR 1993 Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. *PNAS* **90** 10553–10557.
- Karasić D, Rosen CJ, Hannan MT, Broe KE, Dawson-Hughes B, Gagnon DR, Wilson PW, Visser M, Langlois JA, Mohan S *et al.* 2002 Insulin-like growth factor binding proteins 4 and 5 and bone mineral density in elderly men and women. *Calcified Tissue International* **71** 323–328.
- Kassem M, Okazaki R, De Leon D, Harris SA, Robinson JA, Spelsberg TC, Conover CA & Riggs BL 1996 Potential mechanism of estrogen-mediated decrease in bone formation: estrogen increases production of inhibitory insulin-like growth factor-binding protein-4. *Proceedings of the Association of American Physicians* **108** 155–164.
- Kassem M, Brixen K, Mosekilde L, Blum WF & Flyvbjerg A 1998 Effects of growth hormone treatment on serum levels of insulin-like growth factors (IGFs) and IGF binding proteins 1–4 in postmenopausal women. *Clinical Endocrinology* **49** 747–756.
- Katz LE, Bhala A, Camron E, Nunn SE, Hintz RL & Cohen P 1997 IGF-II, IGF-binding proteins and IGF receptors in pancreatic beta-cell lines. *Journal of Endocrinology* **152** 455–464.
- Kelley KM, Oh Y, Gargosky SE, Gucsev Z, Matsumoto T, Hwa V, Ng L, Simpson DM & Rosenfeld RG 1996 Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. *International Journal of Biochemistry and Cell Biology* **28** 619–637.
- Kniss DA, Shubert PJ, Zimmerman PD, Landon MB & Gabbe SG 1994 Insulin-like growth factors. Their regulation of glucose and amino acid transport in placental trophoblasts isolated from first-trimester chorionic villi. *Journal of Reproductive Medicine* **39** 249–256.
- Knudtson KL, Boes M, Sandra A, Dake BL, Booth BA & Bar RS 2001 Distribution of chimeric IGF binding protein (IGFBP)-3 and IGFBP-4 in the rat heart: importance of C-terminal basic region. *Endocrinology* **142** 3749–3755.
- Kudo Y, Iwashita M, Itatsu S, Iguchi T & Takeda Y 1996 Regulation of insulin-like growth factor-binding protein-4 protease activity by estrogen and parathyroid hormone in SaOS-2 cells: implications for the pathogenesis of postmenopausal osteoporosis. *Journal of Endocrinology* **150** 223–229.
- Kudo Y, Iwashita M, Iguchi T, Takeda Y, Hizuka N, Takano K & Muraki T 1997 Estrogen and parathyroid hormone regulate insulin-like growth factor binding protein-4 in SaOS-2 cells. *Life Sciences* **61** 165–170.

- Kuemmerle JF & Teng B 2000 Regulation of IGFBP-4 levels in human intestinal muscle by an IGF-I-activated, confluence-dependent protease. *American Journal of Physiology* **279** G975–G982.
- Kveiborg M, Flyvbjerg A, Eriksen EF & Kassem M 2001 1,25-Dihydroxyvitamin D3 stimulates the production of insulin-like growth factor-binding proteins-2, -3 and -4 in human bone marrow stromal cells. *European Journal of Endocrinology* **144** 549–557.
- Landale EC, Strong DD, Mohan S & Baylink DJ 1995 Sequence comparison and predicted structure for the four exon-encoded regions of human insulin-like growth factor binding protein 4. *Growth Factors* **12** 245–250.
- LaTour D, Mohan S, Linkhart TA, Baylink DJ & Strong DD 1990 Inhibitory insulin-like growth factor-binding protein: cloning, complete sequence, and physiological regulation. *Molecular Endocrinology* **4** 1806–1814.
- Laursen LS, Overgaard MT, Soe R, Boldt HB, Sottrup-Jensen L, Giudice LC, Conover CA & Oxvig C 2001 Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. *FEBS Letters* **504** 36–40.
- Laursen LS, Overgaard MT, Nielsen CG, Boldt HB, Hopmann KH, Conover CA, Sottrup-Jensen L, Giudice LC & Oxvig C 2002a Substrate specificity of the metalloproteinase pregnancy-associated plasma protein-A (PAPP-A) assessed by mutagenesis and analysis of synthetic peptides: substrate residues distant from the scissile bond are critical for proteolysis. *Biochemical Journal* **367** 31–40.
- Laursen LS, Overgaard MT, Weyer K, Boldt HB, Ebbesen P, Christiansen M, Sottrup-Jensen L, Giudice LC & Oxvig C 2002b Cell surface targeting of pregnancy-associated plasma protein A proteolytic activity. Reversible adhesion is mediated by two neighboring short consensus repeats. *Journal of Biological Chemistry* **277** 47225–47234.
- Lawrence JB, Bale LK, Haddad TC, Clarkson JT & Conover CA 1999a Characterization and partial purification of the insulin-like growth factor (IGF)-dependent IGF binding protein-4-specific protease from human fibroblast conditioned media. *Growth Hormone and IGF Research* **9** 25–34.
- Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, Yates JR III & Conover CA 1999b The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *PNAS* **96** 3149–3153.
- Leal SM, Liu Q, Huang SS & Huang JS 1997 The type V transforming growth factor beta receptor is the putative insulin-like growth factor-binding protein 3 receptor. *Journal of Biological Chemistry* **272** 20572–20576.
- Lee HG, Vega RA, Phung LT, Matsunaga N, Kuwayama H & Hidari H 2000 The effect of growth hormone-releasing peptide-2 (KP102) administration on plasma insulin-like growth factor (IGF)-1 and IGF-binding proteins in Holstein steers on different planes of nutrition. *Domestic Animal Endocrinology* **18** 293–308.
- LeRoith D 2000 Insulin-like growth factor I receptor signaling – overlapping or redundant pathways? *Endocrinology* **141** 1287–1288.
- LeRoith D, Werner H, Beitner-Johnson D & Roberts CT Jr 1995 Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocrine Reviews* **16** 143–163.
- Lewis MI, Horvitz GD, Clemmons DR & Fournier M 2002 Role of IGF-I and IGF-binding proteins within diaphragm muscle in modulating the effects of nandrolone. *American Journal of Physiology* **282** E483–E490.
- Li YM, Arkins S, McCusker RH Jr, Donovan SM, Liu Q, Jayaraman S, Dantzer R & Kelley KW 1996 Macrophages synthesize and secrete a 25-kilodalton protein that binds insulin-like growth factor-I. *Journal of Immunology* **156** 64–72.
- Lin TM, Galbert SP, Kiefer D, Spellacy WN & Gall S 1974 Characterization of four human pregnancy-associated plasma proteins. *American Journal of Obstetrics and Gynecology* **118** 223–236.
- Lindberg MK, Moverare S, Eriksson AL, Skrtic S, Gao H, Dahlman-Wright K, Gustafsson JA & Ohlsson C 2002 Identification of estrogen-regulated genes of potential importance for the regulation of trabecular bone mineral density. *Journal of Bone and Mineral Research* **17** 2183–2195.
- Lindenbergh-Kortleve DJ, Rosato RR, van Neck JW, Nauta J, van Kleffens M, Groffen C, Zwarthoff EC & Drop SL 1997 Gene expression of the insulin-like growth factor system during mouse kidney development. *Molecular and Cellular Endocrinology* **132** 81–91.
- Liu JL, Grinberg A, Westphal H, Sauer B, Accili D, Karas M & LeRoith D 1998 Insulin-like growth factor-I affects perinatal lethality and postnatal development in a gene dosage-dependent manner: manipulation using the Cre/loxP system in transgenic mice. *Molecular Endocrinology* **12** 1452–1462.
- Liu JP, Baker J, Perkins AS, Robertson EJ & Efstratiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (IGF-1) and type 1 IGF receptor (IGF1R). *Cell* **75** 59–72.
- Maile LA & Holly JM 1999 Insulin-like growth factor binding protein (IGFBP) proteolysis: occurrence, identification, role and regulation. *Growth Hormone and IGF Research* **9** 85–95.
- Mason HD, Cwyfan-Hughes S, Holly JM & Franks S 1998 Potent inhibition of human ovarian steroidogenesis by insulin-like growth factor binding protein-4 (IGFBP-4). *Journal of Clinical Endocrinology and Metabolism* **83** 284–287.
- Matsell DG, Delhanty PJ, Stepaniuk O, Goodyear C & Han VK 1994 Expression of insulin-like growth factor and binding protein genes during nephrogenesis. *Kidney International* **46** 1031–1042.
- Mazebourg S, Zapf J, Bar RS, Brigstock DR & Monget P 2000 Insulin-like growth factor (IGF)-binding protein-4 proteolytic degradation in bovine, equine, and porcine preovulatory follicles: regulation by IGFs and heparin-binding domain-containing peptides. *Biology of Reproduction* **63** 390–400.
- Mazebourg S, Overgaard MT, Oxvig C, Christiansen M, Conover CA, Laurendeau I, Vidaud M, Tosser-Klopp G, Zapf J & Monget P 2001 Pregnancy-associated plasma protein-A (PAPP-A) in ovine, bovine, porcine, and equine ovarian follicles: involvement in IGF binding protein-4 proteolytic degradation and mRNA expression during follicular development. *Endocrinology* **142** 5243–5253.
- McCusker RH & Clemmons DR 1998 Role for cyclic adenosine monophosphate in modulating insulin-like growth factor binding protein secretion by muscle cells. *Journal of Cellular Physiology* **174** 293–300.
- McKinnon T, Chakraborty C, Gleeson LM, Chidiac P & Lala PK 2001 Stimulation of human extravillous trophoblast migration by IGF-II is mediated by IGF type 2 receptor involving inhibitory G protein(s) and phosphorylation of MAPK. *Journal of Clinical Endocrinology and Metabolism* **86** 3665–3674.
- Michell NP, Dent S, Langman MJ & Eggo MC 1997 Insulin-like growth factor binding proteins as mediators of IGF-I effects on colon cancer cell proliferation. *Growth Factors* **14** 269–277.
- Milne M, Quail JM, Rosen CJ & Baran DT 2001 Insulin-like growth factor binding proteins in femoral and vertebral bone marrow stromal cells: expression and regulation by thyroid hormone and dexamethasone. *Journal of Cellular Biochemistry* **81** 229–240.
- Miyakoshi N, Richman C, Qin X, Baylink DJ & Mohan S 1999 Effects of recombinant insulin-like growth factor-binding protein-4 on bone formation parameters in mice. *Endocrinology* **140** 5719–5728.
- Miyakoshi N, Qin X, Kasukawa Y, Richman C, Srivastava AK, Baylink DJ & Mohan S 2001 Systemic administration of insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) increases bone formation parameters in mice by increasing IGF bioavailability via an IGFBP-4 protease-dependent mechanism. *Endocrinology* **142** 2641–2648.
- Mohan S & Baylink DJ 2002 IGF-binding proteins are multifunctional and act via IGF-dependent and -independent mechanisms. *Journal of Endocrinology* **175** 19–31.

- Mohan S, Bautista CM, Wergedal J & Baylink DJ 1989 Isolation of an inhibitory insulin-like growth factor (IGF) binding protein from bone cell-conditioned medium: a potential local regulator of IGF action. *PNAS* **86** 8338–8342.
- Mohan S, Farley JR & Baylink DJ 1995a Age-related changes in IGFBP-4 and IGFBP-5 levels in human serum and bone: implications for bone loss with aging. *Progress in Growth Factor Research* **6** 465–473.
- Mohan S, Nakao Y, Honda Y, Landale E, Leser U, Dony C, Lang K & Baylink DJ 1995b Studies on the mechanisms by which insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) and IGFBP-5 modulate IGF actions in bone cells. *Journal of Biological Chemistry* **270** 20424–20431.
- Mohan S, Baylink DJ & Pettis JL 1996 Insulin-like growth factor (IGF)-binding proteins in serum – do they have additional roles besides modulating the endocrine IGF actions? *Journal of Clinical Endocrinology and Metabolism* **81** 3817–3820.
- Monget P, Fabre S, Mulsant P, Lecerf F, Elsen JM, Mazerbourg S, Pisselet C & Monniaux D 2002 Regulation of ovarian folliculogenesis by IGF and BMP system in domestic animals. *Domestic Animal Endocrinology* **23** 139–154.
- Monget P, Mazerbourg S, Delpuech T, Maurel MC, Maniere S, Zapf J, Lalmanach G, Oxvig C & Overgaard MT 2003 Pregnancy-associated plasma protein-A is involved in insulin-like growth factor binding protein-2 (IGFBP-2) proteolytic degradation in bovine and porcine preovulatory follicles: identification of cleavage site and characterization of IGFBP-2 degradation. *Biology of Reproduction* **68** 77–86.
- Murphy LJ 1998 Insulin-like growth factor-binding proteins: functional diversity or redundancy? *Journal of Molecular Endocrinology* **21** 97–107.
- Nestler JE 1987 Modulation of aromatase and P450 cholesterol side-chain cleavage enzyme activities of human placental cytotrophoblasts by insulin and insulin-like growth factor I. *Endocrinology* **121** 1845–1852.
- Nestler JE 1990 Insulin-like growth factor II is a potent inhibitor of the aromatase activity of human placental cytotrophoblasts. *Endocrinology* **127** 2064–2070.
- Neumann GM & Bach LA 1999 The N-terminal disulfide linkages of human insulin-like growth factor-binding protein-6 (hIGFBP-6) and hIGFBP-1 are different as determined by mass spectrometry. *Journal of Biological Chemistry* **274** 14587–14594.
- Nickerson T & Huynh H 1999 Vitamin D analogue EB1089-induced prostate regression is associated with increased gene expression of insulin-like growth factor binding proteins. *Journal of Endocrinology* **160** 223–229.
- Ninh NX, Maiter D, Laue P, Chrzanowska B, Underwood LE, Ketelslegers JM & Thissen JP 1998 Continuous administration of growth hormone does not prevent the decrease of IGF-I gene expression in zinc-deprived rats despite normalization of liver GH binding. *Growth Hormone and IGF Research* **8** 465–472.
- Noll K, Wegmann BR, Havemann K & Jaques G 1996 Insulin-like growth factors stimulate the release of insulin-like growth factor-binding protein-3 (IGFBP-3) and degradation of IGFBP-4 in nonsmall cell lung cancer cell lines. *Journal of Clinical Endocrinology and Metabolism* **81** 2653–2662.
- Oh Y, Muller HL, Lamson G & Rosenfeld RG 1993 Insulin-like growth factor (IGF)-independent action of IGF-binding protein-3 in Hs578T human breast cancer cells. Cell surface binding and growth inhibition. *Journal of Biological Chemistry* **268** 14964–14971.
- Overgaard MT, Haaning J, Boldt HB, Olsen IM, Laursen LS, Christiansen M, Gleich GJ, Sottrup-Jensen L, Conover CA & Oxvig C 2000 Expression of recombinant human pregnancy-associated plasma protein-A and identification of the proform of eosinophil major basic protein as its physiological inhibitor. *Journal of Biological Chemistry* **275** 31128–31133.
- Oxvig C, Sand O, Kristensen T, Gleich GJ & Sottrup-Jensen L 1993 Circulating human pregnancy-associated plasma protein-A is disulfide-bridged to the proform of eosinophil major basic protein. *Journal of Biological Chemistry* **268** 12243–12246.
- Perks CM, Bowen S, Gill ZP, Newcomb PV & Holly JM 1999 Differential IGF-independent effects of insulin-like growth factor binding proteins (1–6) on apoptosis of breast epithelial cells. *Journal of Cellular Biochemistry* **75** 652–664.
- Piferer F, Li D, Shimasaki S & Erickson GF 1997 Transforming growth factor- α stimulates insulin-like growth factor binding protein-4 (IGFBP-4) expression and blocks follicle-stimulating hormone regulation of IGFBP-4 production in rat granulosa cells. *Molecular and Cellular Endocrinology* **133** 9–17.
- Poretsky L, Cataldo NA, Rosenwaks Z & Giudice LC 1999 The insulin-related ovarian regulatory system in health and disease. *Endocrine Reviews* **20** 535–582.
- Price GJ, Berka JL, Edmondson SR, Werther GA & Bach LA 1995 Localization of mRNAs for insulin-like growth factor binding proteins 1 to 6 in rat kidney. *Kidney International* **48** 402–411.
- Price WA 1999 Peptide growth factors regulate insulin-like growth factor binding protein production by fetal rat lung fibroblasts. *American Journal of Respiratory Cell and Molecular Biology* **20** 332–341.
- Price WA 2001 PDGF-BB regulates IGF-mediated IGFBP-4 proteolysis in fetal lung fibroblasts. *Experimental Lung Research* **27** 655–674.
- Price WA, Moats-Staats BM & Stiles AD 1995 Insulin-like growth factor-I (IGF-I) regulates IGFBP-3 and IGFBP-4 by multiple mechanisms in A549 human adenocarcinoma cells. *American Journal of Respiratory Cell and Molecular Biology* **13** 466–476.
- Price WA, Moats-Staats BM & Stiles AD 2002 Pro- and anti-inflammatory cytokines regulate insulin-like growth factor binding protein production by fetal rat lung fibroblasts. *American Journal of Respiratory Cell and Molecular Biology* **26** 283–289.
- Putowski L, Rohan RM, Choi DS, Scherzer WJ, Ricciarelli E, Mordacq J, Mayo KE & Adashi EY 1997 Rat ovarian insulin-like growth factor binding protein-4: a hormone-dependent granulosa cell-derived antigonadotropin. *Journal of the Society for Gynecologic Investigation* **4** 144–151.
- Qin C, Singh P & Safe S 1999 Transcriptional activation of insulin-like growth factor-binding protein-4 by 17 β -estradiol in MCF-7 cells: role of estrogen receptor-Sp1 complexes. *Endocrinology* **140** 2501–2508.
- Qin X, Strong DD, Baylink DJ & Mohan S 1998 Structure–function analysis of the human insulin-like growth factor binding protein-4. *Journal of Biological Chemistry* **273** 23509–23516.
- Qin X, Byun D, Strong DD, Baylink DJ & Mohan S 1999 Studies on the role of human insulin-like growth factor-II (IGF-II)-dependent IGF binding protein (hIGFBP)-4 protease in human osteoblasts using protease-resistant IGFBP-4 analogs. *Journal of Bone and Mineral Research* **14** 2079–2088.
- Qin X, Byun D, Lau KW, Baylink DJ & Mohan S 2000 Evidence that the interaction between insulin-like growth factor (IGF)-II and IGF binding protein (IGFBP)-4 is essential for the action of the IGF-II-dependent IGFBP-4 protease. *Archives of Biochemistry and Biophysics* **379** 209–216.
- Qin X, Sexton C, Byun D, Strong DD, Baylink DJ & Mohan S 2002 Differential regulation of pregnancy associated plasma protein (PAPP)-A during pregnancy in human and mouse. *Growth Hormone and IGF Research* **12** 359–366.
- Rajaram S, Baylink DJ & Mohan S 1997 Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. *Endocrine Reviews* **18** 801–831.
- Rechler MM 1993 Insulin-like growth factor binding proteins. *Vitamins and Hormones* **47** 1–114.
- Richardson RL, Hausman GJ & Wright JT 1998 Growth factor regulation of insulin-like growth factor (IGF) binding proteins (IGFBP) and preadipocyte differentiation in porcine stromal-vascular cell cultures. *Growth, Development, and Aging* **62** 3–12.
- Rivera GM & Fortune JE 2003 Selection of the dominant follicle and insulin-like growth factor (IGF)-binding proteins: evidence that

- pregnancy-associated plasma protein A contributes to proteolysis of IGF-binding protein 5 in bovine follicular fluid. *Endocrinology* **144** 437–446.
- Rooman RP, De Beeck LO, Martin M, Van Doorn J, Mohan S & Du Caju MV 2002 IGF-I, IGF-II, 'free' IGF-I and IGF-binding proteins-2 to -6 during high-dose oestrogen treatment in constitutionally tall girls. *European Journal of Endocrinology* **146** 823–829.
- Rosato R, Lindenbergh-Kortleve D, Neck J, Drop S & Jahn G 2002 Effect of chronic thyroxine treatment on IGF-I, IGF-II and IGF-binding protein expression in mammary gland and liver during pregnancy and early lactation in rats. *European Journal of Endocrinology* **146** 729–739.
- Russo VC, Rekaris G, Baker NL, Bach LA & Werther GA 1999 Basic fibroblast growth factor induces proteolysis of secreted and cell membrane-associated insulin-like growth factor binding protein-2 in human neuroblastoma cells. *Endocrinology* **140** 3082–3090.
- San Roman GA & Magoffin DA 1993 Insulin-like growth factor-binding proteins in healthy and atretic follicles during natural menstrual cycles. *Journal of Clinical Endocrinology and Metabolism* **76** 625–632.
- Scharf JG, Schmidt-Sandte W, Pahernik SA, Ramadori G, Bräulke T & Hartmann H 1998 Characterization of the insulin-like growth factor axis in a human hepatoma cell line (PLC). *Carcinogenesis* **19** 2121–2128.
- Schiltz PM, Mohan S & Baylink DJ 1993 Insulin-like growth factor binding protein-4 inhibits both basal and IGF-mediated chick pelvic cartilage growth *in vitro*. *Journal of Bone and Mineral Research* **8** 391–396.
- Schneider MR, Wolf E, Hoefflich A & Lahm H 2002 IGF-binding protein-5: flexible player in the IGF system and effector on its own. *Journal of Endocrinology* **172** 423–440.
- Schuller AG, van Neck JW, Lindenbergh-Kortleve DJ, Groffen C, de Jong I, Zwarthoff EC & Drop SL 1993 Gene expression of the IGF binding proteins during post-implantation embryogenesis of the mouse; comparison with the expression of IGF-I and -II and their receptors in rodent and human. *Advances in Experimental Medicine and Biology* **343** 267–277.
- Shimasaki S, Uchiyama F, Shimonaka M & Ling N 1990 Molecular cloning of the cDNAs encoding a novel insulin-like growth factor-binding protein from rat and human. *Molecular Endocrinology* **4** 1451–1458.
- Shoubridge CA, Steeb CB & Read LC 2001 IGFBP mRNA expression in small intestine of rat during postnatal development. *American Journal of Physiology* **281** G1378–G1384.
- Singh P, Dai B, Dhruva B & Widen SG 1994 Episomal expression of sense and antisense insulin-like growth factor (IGF)-binding protein-4 complementary DNA alters the mitogenic response of a human colon cancer cell line (HT-29) by mechanisms that are independent of and dependent upon IGF-I. *Cancer Research* **54** 6563–6570.
- Smith EP, Kamyar A, Niu W, Wang J, Cercek B, Chernauek SD & Fagin JA 2001 IGF-binding protein-4 expression and IGF-binding protein-4 protease activity are regulated coordinately in smooth muscle during postnatal development and after vascular injury. *Endocrinology* **142** 4420–4427.
- Soe R, Overgaard MT, Thomsen AR, Laursen LS, Olsen IM, Sottorp-Jensen L, Haaning J, Giudice LC, Conover CA & Oxvig C 2002 Expression of recombinant murine pregnancy-associated plasma protein-A (PAPP-A) and a novel variant (PAPP-Ai) with differential proteolytic activity. *European Journal of Biochemistry* **269** 2247–2256.
- Soto L, Martin AI, Millan S, Vara E & Lopez-Calderon A 1998 Effects of endotoxin lipopolysaccharide administration on the somatotrophic axis. *Journal of Endocrinology* **159** 239–246.
- Spencer EM & Chan K 1995 A 3-dimensional model for the insulin-like growth factor binding proteins (IGFBPs); supporting evidence using the structural determinants of the IGF binding site on IGFBP-3. *Progress in Growth Factor Research* **6** 209–214.
- Srinivasan N, Edwall D, Linkhart TA, Baylink DJ & Mohan S 1996 Insulin-like growth factor-binding protein-6 produced by human PC-3 prostate cancer cells: isolation, characterization and its biological action. *Journal of Endocrinology* **149** 297–303.
- Standker L, Bräulke T, Mark S, Mostafavi H, Meyer M, Honing S, Gimenez-Gallego G & Forssmann WG 2000 Partial IGF affinity of circulating N- and C-terminal fragments of human insulin-like growth factor binding protein-4 (IGFBP-4) and the disulfide bonding pattern of the C-terminal IGFBP-4 domain. *Biochemistry* **39** 5082–5088.
- Stewart CE & Rotwein P 1996 Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. *Physiological Reviews* **76** 1005–1026.
- Sun IY, Overgaard MT, Oxvig C & Giudice LC 2002 Pregnancy-associated plasma protein A proteolytic activity is associated with the human placental trophoblast cell membrane. *Journal of Clinical Endocrinology and Metabolism* **87** 5235–5240.
- Thomas LN, Wright AS, Lazier CB, Cohen P & Rittmaster RS 2000 Prostatic involution in men taking finasteride is associated with elevated levels of insulin-like growth factor-binding proteins (IGFBPs)-2, -4, and -5. *Prostate* **42** 203–210.
- Thomas T, Gori F, Spelsberg TC, Khosla S, Riggs BL & Conover CA 1999 Response of bipotential human marrow stromal cells to insulin-like growth factors: effect on binding protein production, proliferation, and commitment to osteoblasts and adipocytes. *Endocrinology* **140** 5036–5044.
- Tonin P, Ehrenborg E, Lenoir G, Feunteun J, Lynch H, Morgan K, Zazzi H, Vivier A, Pollak M, Huynh H *et al.* 1993 The human insulin-like growth factor-binding protein 4 gene maps to chromosome region 17q12-q21.1 and is close to the gene for hereditary breast-ovarian cancer. *Genomics* **18** 414–417.
- Ui M, Shimonaka M, Shimasaki S & Ling N 1989 An insulin-like growth factor-binding protein in ovarian follicular fluid blocks follicle-stimulating hormone-stimulated steroid production by ovarian granulosa cells. *Endocrinology* **125** 912–916.
- Ullinski T, Mohan S, Kiepe D, Blum WF, Wingen AM, Mehls O & Tönshoff B 2000 Serum insulin-like growth factor binding protein (IGFBP)-4 and IGFBP-5 in children with chronic renal failure: relationship to growth and glomerular filtration rate. The European Study Group for Nutritional Treatment of Chronic Renal Failure in Childhood. German Study Group for Growth Hormone Treatment in Chronic Renal Failure. *Pediatric Nephrology* **14** 589–597.
- Van Doorn J, Cornelissen AJ & Buul-Offers SC 2001 Plasma levels of insulin-like growth factor binding protein-4 (IGFBP-4) under normal and pathological conditions. *Clinical Endocrinology* **54** 655–664.
- van Kleffens M, Groffen CA, Dits NF, Lindenbergh-Kortleve DJ, Schuller AG, Bradshaw SL, Pintar JE, Zwarthoff EC, Drop SL & van Neck JW 1999 Generation of antisera to mouse insulin-like growth factor binding proteins (IGFBP)-1 to -6: comparison of IGFBP protein and messenger ribonucleic acid localization in the mouse embryo. *Endocrinology* **140** 5944–5952.
- van Neck JW, Dits NF, Cingel V, Hoppenbrouwers IA, Drop SL & Flyvbjerg A 2000 Dose-response effects of a new growth hormone receptor antagonist (B2036-PEG) on circulating, hepatic and renal expression of the growth hormone/insulin-like growth factor system in adult mice. *Journal of Endocrinology* **167** 295–303.
- Voci A, Demori, Bottazzi C & Fugassa E 2001 Regulation of renal growth and IGFBP-4 expression by triiodothyronine during rat development. *Hormone and Metabolism Research* **33** 256–262.
- Wagner KJ, Patek CE, Miles C, Christie S, Brookes AJ & Hooper ML 2001 Truncation of WT1 results in downregulation of cyclin G1 and IGFBP-4 expression. *Biochemical and Biophysical Research Communications* **287** 977–982.

- Wang J, Niu W, Nikiforov Y, Naito S, Chernauek S, Witte D, LeRoith D, Strauch A & Fagin JA 1997 Targeted overexpression of IGF-I evokes distinct patterns of organ remodeling in smooth muscle cell tissue beds of transgenic mice. *Journal of Clinical Investigation* **100** 1425–1439.
- Wang J, Niu W, Witte DP, Chernauek SD, Nikiforov YE, Clemens TL, Sharifi B, Strauch AR & Fagin JA 1998 Overexpression of insulin-like growth factor-binding protein-4 (IGFBP-4) in smooth muscle cells of transgenic mice through a smooth muscle alpha-actin-IGFBP-4 fusion gene induces smooth muscle hypoplasia. *Endocrinology* **139** 2605–2614.
- Wang JF, Hampton B, Mehlman T, Burgess WH & Rechler MM 1988 Isolation of a biologically active fragment from the carboxy terminus of the fetal rat binding protein for insulin-like growth factors. *Biochemical and Biophysical Research Communications* **157** 718–726.
- Westergaard JG, Chemnitz J, Teisner B, Poulsen HK, Ipsen L, Beck B & Grudzinskas JG 1983 Pregnancy-associated plasma protein A: a possible marker in the classification and prenatal diagnosis of Cornelia de Lange syndrome. *Prenatal Diagnosis* **3** 225–232.
- Wetterau LA, Moore MG, Lee KW, Shim ML & Cohen P 1999 Novel aspects of the insulin-like growth factor binding proteins. *Molecular Genetics and Metabolism* **68** 161–181.
- Wright RJ, Holly JM, Galea R, Brincat M & Mason HD 2002 Insulin-like growth factor (IGF)-independent effects of IGF binding protein-4 on human granulosa cell steroidogenesis. *Biology of Reproduction* **67** 776–781.
- Yeh LC, Adamo ML, Kitten AM, Olson MS & Lee JC 1996 Osteogenic protein-1-mediated insulin-like growth factor gene expression in primary cultures of rat osteoblastic cells. *Endocrinology* **137** 1921–1931.
- Yi HK, Hwang PH, Yang DH, Kang CW & Lee DY 2001 Expression of the insulin-like growth factors (IGFs) and the IGF-binding proteins (IGFBPs) in human gastric cancer cells. *European Journal of Cancer* **37** 2257–2263.
- Zazzi H, Nikoshkov A, Hall K & Luthman H 1998 Structure and transcription regulation of the human insulin-like growth factor binding protein 4 gene (IGFBP4). *Genomics* **49** 401–410.
- Zhang M, Smith EP, Kuroda H, Banach W, Chernauek SD & Fagin JA 2002 Targeted expression of a protease-resistant IGFBP-4 mutant in smooth muscle of transgenic mice results in IGFBP-4 stabilization and smooth muscle hypotrophy. *Journal of Biological Chemistry* **277** 21285–21290.
- Zhou J & Bondy C 1993 Anatomy of the human ovarian insulin-like growth factor system. *Biology of Reproduction* **48** 467–482.

Received in final form 13 March 2003

Accepted 28 April 2003

Made available online as an Accepted Preprint
6 May 2003